12

AD

# TOXICITY OF TNT WASTEWATERS TO AQUATIC ORGANISMS

Volume II

## Acute Toxicity of Condensate Wastewater and 2,4-Dinitrotoluene

By

DAVID H. W. LIU
RONALD J. SPANGGORD
HOWARD C. BAILEY
HAROLD S. JAVITZ
DAVID C. L. JONES

MARCH 1983
~~June 1984~~

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK, FREDERICK, MD 21701

Contract DAMD17-75-C-5056
SRI International
333 Ravenswood Avenue
Menlo Park, CA 94025

Project Officer: J. Gareth Pearson
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, MD 21701





DoD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

# REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

| | |
|---|---|
| 1. REPORT NUMBER | 2. GOVT ACCESSION NO. AD A141 2 |
| 3. RECIPIENT'S CATALOG NUMBER 145 | |
| 4. TITLE (and Subtitle) TOXICITY OF TNT WASTEWATERS TO AQUATIC ORGANISMS Volume II - Acute Toxicity of Condensate Wastewater and 2,4-Dinitrotoluene | 5. TYPE OF REPORT & PERIOD COVERED Final Report, 15 April 1975 to 15 December 1978 |
| | 6. PERFORMING ORG. REPORT NUMBER LSU-4262 |
| 7. AUTHOR(s) David H. W. Liu, Ronald J. Spanggord, Howard C. Bailey, Harold S. Javitz, and David C. L. Jones | 8. CONTRACT OR GRANT NUMBER(s) DAMD 17-75-C-5056 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS SRI International 333 Ravenswood Avenue Menlo Park, CA 94025 | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A.3E162720A835.AA.045 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Fort Detrick, Frederick, MD 21701 | 12. REPORT DATE March 1983 |
| | 13. NUMBER OF PAGES 70 |
| 14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) U.S. Army Medical Bioengineering Research and Development Laboratory | 15. SECURITY CLASS. (of this report) Unclassified |
| | 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE N/A |

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

| | | |
|---|---|---|
| Acute toxicity | Photolysis | Red water |
| Incipient LC50 | Water quality | Condensate wastewater |
| Fish | Toxic interactions | Synthetic condensate wastewater |
| Invertebrates | Bioconcentration | Toluene |
| Algae | Fish eggs and larvae | 2-Nitrotoluene |

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Condensate wastewater is a distillation product of red water, which is produced at U.S. Army ammunition plants during the continuous manufacture of 2,4,6-trinitrotoluene (TNT). Condensate wastewater is composed primarily of nitroaromatic by-products of TNT manufacture. At least 30 organic compounds have been identified in the wastewater that are attributable to TNT production. The major component is 2,4-dinitrotoluene (DNT). Acute toxicity tests on red water, condensate wastewater, and 2,4-DNT produced 96-hour LC50s of 360 and 185 mg/L as total dissolved solids for red

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

19. KEY WORDS (Continued)

| | | |
|---|---|---|
| 3-Nitrotoluene | 4-Amino-2-nitrotoluene | 2,3,6-Trinitrobenzene |
| 4-Nitrotoluene | 3-Methyl-2-nitrophenol | 3,5-Dinitrotoluene |
| 3-Nitrobenzonitrile | 5-Methyl-2-nitrophenol | 3,5-Dinitroaniline |
| 4-Nitrobenzonitrile | 1,3-Dinitrobenzene | 1,5-Dimethyl-2,4-dinitrobenzene |
| 2-Amino-4-nitrotoluene | 2,3-Dinitrotoluene | 2-Amino-3,6-dinitrotoluene |
| 2-Amino-6-nitrotoluene | 2,5-Dinitrotoluene | 2-Amino-4,6-dinitrotoluene |
| 3-Amino-4-nitrotoluene | 2,6-Dinitrotoluene | 3-Amino-2,4-dinitrotoluene |
| 5-Amino-2,4-dinitrotoluene | 3,4-Dinitrotoluene | 3-Amino-2,6-dinitrotoluene |
| 2,4-Dinitro-5-methylphenol | 1,3,5-Trinitrobenzene | 4-Amino-2,6-dinitrotoluene |
| | | 4-Amino-3,5-dinitrotoluene |
| | | 2,4,6-Trinitrotoluene |

20 ABSTRACT (Continued)

water and condensate wastewater, respectively, and 31.4 mg/L for 2,4-DNT in fathead minnows (Pimephales promelas). Exposure of condensate wastewater and 2,4-DNT to filtered UV light reduced their acute toxicity by a factor of about 2, but the same treatment did not affect the acute toxicity of red water.

Benzene extracts of red water and condensate wastewater were more toxic than the remaining aqueous fractions. This suggested that the nonpolar components contribute more to the toxicity of these wastewaters than the polar components.

Acute toxicity tests performed on 30 of the organic components of condensate wastewater revealed two with 96-hour LC50s of less than 1.0 mg/L in fathead minnows. These components were 2,3,6-TNT (96-hr LC50, 0.11 mg/L) and 2-amino-3,6-DNT (96-hr LC50, 0.9 mg/L). These components and 13 others were more toxic to the minnows than to the water flea (Daphnia magna).

Toxic unit values, computed for each compound by dividing its average measured concentration in condensate wastewater by its 96-hour LC50 in minnows and 48-hour EC50 in D. magna, revealed that 2,3,6-TNT may contribute most to the acute toxicity of the wastewater to minnows, even though its average wastewater concentration is only 0.8 percent of the total average concentration of all 30 compounds.

Acute toxicity tests on several synthetic condensate wastewater formulations and on 2,4-DNT showed the bluegill (Lepomis macrochirus) and the rainbow trout (Salmo gairdnerii) to be more sensitive to both materials than the fathead minnow and the channel catfish (Ictalurus punctatus). The 2,4-DNT was more toxic to midge (Tanytarsus dissimilis) than to the water flea (D. magna), scud (Hyalella azteca), and worm (Lumbriculus variegatus); but T. dissimilis was the least sensitive invertebrate species to synthetic condensate wastewater.

Fourteen-day static algal assays, performed with Selenastrum capricornutum, Anabaena flos-aquae, Microcystis aeruginosa, and Navicula pelliculosa, showed 2,4-DNT to be most toxic to M. aeruginosa and synthetic condensate wastewater to be most toxic to S. capricornutum. Photoirradiation of the synthetic wastewater had no appreciable effect on the toxicity of the wastewater to S. capriconutum, the only algal species tested.

Neither water temperature (15 to 25°C), pH (6 to 8), nor hardness (40 to 250 mg/L) had appreciable effects on the acute toxicity to bluegills of 2,4,-DNT or synthetic condensate wastewater.

19. KEY WORDS (Continued)

20 ABSTRACT (Continued)

Synthetic condensate wastewater was more toxic to two-day-old minnow fry than to minnow embryos and older fry. Exposure of the wastewater to filtered UV light reduced its toxicity to all of the life stages tested, but did not change the relative sensitivity of the stages.

Four-day bioconcentration factors (BCF) computed for 2,4-DNT from four-day bioconcentration test data were less than 100 in bluegills, D. magna, and L. variegatus, but 2100 to 2500 in the alga S. capricornutum. In bluegills, depuration of the radio-labelled material was very rapid. Steady-state BCFs, computed from estimated Log P (octanol-water partition coefficient) values for the 30 organic components of condensate wastewater, ranged from 1.18 to 102.8. The BCF values, applicable to fish with a fat content of about 8 percent, suggest that none of the components has a high propensity to bioconcentrate in fish.

For synthetic condensate wastewater, the incipient LC50s, determined under flow-through test conditions using minnows, bluegills, catfish, and trout, ranged from 6.3 to 24.7 mg/L for 2,4-DNT and 2.3 to 7.3 mg/L for synthetic condensate wastewater. For D. magna, the incipient LC50s for 2,4-DNT and the wastewater were 5.4 and 8.0 mg/L, respectively. For L. variegatus, the respective incipient LC50s were 30.4 and 17.6 mg/L.

It was concluded that the concentrations reported by others of 2,4-DNT in streams near the Volunteer and Joliet Army Ammunition Plants may not be acutely toxic to most of the aquatic animals that inhabit water bodies that receive condensate wastewater; however, the concentrations could inhibit algal growth. Subchronic and chronic toxicity tests on synthetic condensate wastewater and 2,4-DNT were recommended.

## FOREWORD

The U.S. Army Medical Research Development Command, Ft. Detrick, Frederick, MD, has been conducting a research program since 1973 for the purpose of developing the scientific data base necessary for assessing the potential environmental hazards associated with compounds unique to the munitions industry. From these data, criteria will be developed that are qualitative or quantitative estimates of the concentrations of a pollutant in ambient waters which, if not exceeded, should insure the protection of aquatic organisms and human health. These criteria, when compared to actual or estimated environmental concentrations, will form the basis of a hazard assessment. In addition, these criteria will be used to assess the adequacy of current pollution abatement technologies, and thus influence research and development in this area.

This report represents a portion of the data base being developed on 2,4-DNT and the wastewater from continuous production of TNT, and should not be construed as a complete evaluation or as official policy of the U.S. Army Surgeon General.

This work was conducted under the technical control and review of the U.S. Army Medical Bioengineering Research and Development Laboratory represented by J. Gareth Pearson and William H. van der Schalie (Aquatic Toxicology), Jesse J. Barkley, Jr. (Analytical Chemistry), and Jerry W. Highfill (Statistical Analysis).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

## EXECUTIVE SUMMARY

In April 1975, the U.S. Army Medical Research and Development Command contracted with SRI International to conduct a comprehensive laboratory study on the acute, subchronic, and chronic toxicity to aquatic organisms of wastewaters from 2,4,6-trinitrotoluene manufacturing and processing plants. Two kinds of wastewaters were studied: condensate wastewater, which results from treatment of the manufacturing effluent (red water) from the continuous process, and wastewater from COMP B type load, assemble, and pack (LAP) facilities. The results of the entire study are presented in four volumes.

This report presents and discusses the results of acute toxicity and exploratory bioconcentration tests related to condensate wastewater and its organic components with emphasis on 2,4-dinitrotoluene (2,4-DNT). The results of the subchronic and chronic tests are presented in Volume IV. Most of the conclusions about the toxicological properties of condensate wastewater were based on the evaluation of synthetic condensate wastewater (known as condensate water), which was developed and characterized by SRI under a separate contract. Individual components of the wastewater, 2,4-DNT in particular, were evaluated only to gain a better understanding of the toxic properties of the wastewater.

In a preliminary study, conducted on authentic red water and condensate wastewater from the Volunteer Army Ammunition Plant, 96-hour LC50s of 360 and 185 mg/L total dissolved solids were obtained for red water and condensate wastewater, respectively, using the fathead minnow as the test organism. Tests on 2,4-DNT alone produced a 96-hour LC50 of 31.4 mg/L. When exposed to filtered UV light until complete photolysis of 2,4-DNT occurred, the toxicities of condensate wastewater and the solution of 2,4-DNT alone were reduced by a factor of 2. The toxicity of red water was not appreciably affected by photoirradiation. Because red water is the precursor of condensate wastewater and not released from TNT manufacturing plants, we tested it primarily for academic reasons. The results of these preliminary studies indicated that condensate wastewater and 2,4-DNT have relatively low acute toxicity, which decreases when either material is photoirradiated.

To partially identify the toxic segment of the wastewater, we extracted samples of the wastewater with benzene and performed acute toxicity tests on the aqueous and benzene fractions using the fathead minnow and an invertebrate (Daphnia magna). The benzene fraction, containing the nonpolar compounds, was much more acutely toxic than the aqueous fraction.

Acute tests on 30 organic components of condensate wastewater using the fathead minnow and D. magna revealed three compounds with very high toxicity. These compounds were 2,3,6-TNT, 2-amino-3,6-DNT, and 1,3,5-trinitrobenzene. All were more toxic to the minnow than to D. magna, and their 96-hour LC50s ranged from 0.11 mg/L for 2,3,6-TNT to 1.1 mg/L for 1,3,5-trinitrobenzene. Toxic unit values, computed by dividing the average wastewater concentration of each compound by their 48- or 96-hour LC50 showed 2,3,6-TNT to be the only compound whose average concentration in condensate wastewater exceeded its 48- and 96-hour LC50. We inferred that 2,3,6-TNT contributes most to the toxicity of the wastewater. This hypothesis is substantiated

by the fact that the final blend of condensate water (synthetic condensate wastewater) contained 2,3,6-TNT and was more toxic than Blend 1, which did not contain that compound.

In establishing an irradiation endpoint for condensate water, we found that exposure of condensate water to filtered UV light significantly reduced the concentration of all but four of the 28 compounds that we monitored as the sample was being irradiated. The concentration of one of these compounds—1,3,5-trinitrobenzene—actually increased to about two times its initial concentration. The ratio of its initial concentration in condensate water to its 96-hour LC50 in minnows was 0.139, indicating that its contribution to the toxicity of the unirradiated wastewater was insignificant. However, during the irradiation, its concentration increased to a level equal to its 96-hour LC50 in minnows.

In determining the relative sensitivities of four species each of fish, invertebrates, and algae to 2,4-DNT and condensate water, we obtained with the fish species 96-hour LC50s that ranged from 13.5 to 28.5 mg/L for 2,4-DNT and from 7.1 to 22.0 mg/L for condensate water. With the invertebrates, we obtained 48-hour LC50s that ranged from 22.5 to more than 83.2 mg/L for 2,4-DNT and from 22.8 to 37.9 mg/L for condensate water. The bluegill and trout were equally sensitive to both materials and more sensitive than any other animal species evaluated. Microcystis aeruginosa was the most sensitive of the four algae species exposed to 2,4-DNT and exhibited statistically significant inhibition of growth at concentrations as low as 0.5 mg/L. Selenastrum capricornutum showed the highest sensitivity to condensate water, which at 4.8 mg/L caused 98.1 percent inhibition of growth.

Water temperature, pH, and hardness affected the acute toxicity of 2,4-DNT and condensate water; however, the magnitude of effect was not appreciable.

The 2,4-DNT was most acutely toxic to two-day-old minnows and least toxic to the embryos. Condensate water was most toxic to the two- and seven-day-old minnows, which were about equal in sensitivity.

The exploratory four-day bioconcentration tests indicated that ring-labelled 14C-2,4-DNT is not rapidly or extensively taken up from water by invertebrates or fish, and is apparently excreted rapidly by fish. It was extensively sorbed by algae, however. The four-day BCF for 2,4-DNT in algae exceeded 2000. Steady-state BCFs, computed from calculated octanol-water partition coefficients, ranged from 1.18 to 102.8 for the 30 compounds that make up condensate water. We concluded that none of the compounds evaluated has a high propensity to concentrate to hazardous levels in aquatic animals.

The incipient LC50s for 2,4-DNT based on 14-day exposures under flow-through conditions ranged from 5.4 to 30.4 mg/L for the six animal species tested. For condensate water, the incipient LC50s ranged from 2.3 to 17.6 mg/L.

All of the LC50s derived from acute tests performed during this study exceeded the water and sediment concentrations (reported by other investigators) of 2,4-DNT at the Volunteer and Joliet Army Ammunition Plants (VAAP and JAAP) by a factor of at least 3.8. This suggests that the environmental concentrations at these plants may not be acutely toxic to the animal species that inhabit

the receiving waters. However, the algal toxicity data indicate that sediment concentrations of 2,4-DNT at JAAP (which ranged up to 0.6 mg/L) could be high enough to inhibit algal growth.

CONTENTS


FOREWORD . . . . . 1

EXECUTIVE SUMMARY . . . . . 2

LIST OF TABLES . . . . . 7

LIST OF ILLUSTRATIONS . . . . . 10

INTRODUCTION . . . . . 11

BACKGROUND. . . . . 12

- Source of Condensate Wastewater . . . . . 12
- Chemical Composition of Condensate Wastewater . . . . . 12
- Toxicity of Condensate Wastewater to Aquatic Organisms . . . . . 12
- Field Studies . . . . . 15

EXPERIMENTAL APPROACH . . . . . 16

- Phase I: Preliminary Studies . . . . . 16
- Phase II: Acute Toxicity Studies on Condensate Water and Additional Studies on 2,4-DNT . . . . . 16
- Phase III: Definitive Acute Toxicity Studies . . . . . 16

MATERIALS AND EQUIPMENT. . . . . 17

- Materials Evaluated . . . . . 17
- Test Organisms . . . . . 17
- Diluent Water . . . . . 18
- Equipment . . . . . 18

METHODS . . . . . 21

- Acute Toxicity Determination . . . . . 21
- Bioconcentration. . . . . 21
- Preparation of Condensate Water . . . . . 21
- Photoirradiation Methods . . . . . 22
- Preparation of the Aqueous and Benzene Fractions . . . . . 22
- Analytical Chemistry. . . . . 23
- Statistical Methods. . . . . 27

CONTENTS (concluded)


RESULTS AND DISCUSSION . . . . . 30

Phase I: Preliminary Studies . . . . . 30

Task 1: Effect of Photoirradiation on the Acute Toxicity of 2,4-DNT, Red Water, and Condensate Wastewater . . . 30
Task 2: Acute Toxicity of the Aqueous and Benzene Fractions of Red Water, Condensate Wastewater, and a Solution of 50 Percent Photolyzed 2,4-DNT . . . . . 32
Task 3: Acute Toxicity of Selected Organic Components of Condensate Wastewater . . . . . 33

Phase II: Studies on Synthetic Condensate Wastewater (Condensate Water) and Additional Studies on 2,4-DNT . . . . . 37

Task 1: Establishing Photoirradiation End-Point for Condensate Water. . . . . 37
Task 2: Acute Toxicity of Condensate Water and 2,4-DNT to Organisms from Different Trophic Levels . . . . . 41
Task 3: Effects of Water Quality on the Acute Toxicity of 2,4-DNT and Condensate Water to the Bluegill Sunfish . . . . . 47
Task 4: Acute Toxicity of Condensate Water to Selected Early Life Stages of the Fathead Minnow . . . . . 50
Task 5: Exploratory Bioconcentration Studies on 2,4-DNT . . . 51

Phase III: Definitive Acute Toxicity Studies . . . . . 54

Task 1: Determination of Incipient LC50s for 2,4-DNT and Condensate Water . . . . . 54

CONCLUSIONS AND RECOMMENDATIONS . . . . . 61

Conclusions . . . . . 61
Recommendations . . . . . 62

LITERATURE CITED . . . . . 63

DISTRIBUTION LIST . . . . . 66

TABLES


| | | |
|---|---|---|
| 1 | Name and Source of Materials Tested | 19 |
| 2 | Composition of Three Interim Blends and the Final Blend of Condensate Water | 20 |
| 3 | Condensate Melt Analysis Data Summary (for melt produced 3/79) | 26 |
| 4 | Concentrations of 2,4-DNT or 2,4,6-TNT in Samples of Red Water, Condensate Wastewater, and the Stock Solution of 2,4,-DNT before and after Photoirradiation | 30 |
| 5 | Acute Toxicity to Fathead Minnows of Photolyzed and Non-Photolyzed Samples of Red Water, Condensate Wastewater, and 2,4-DNT | 31 |
| 6 | Acute Toxicity of the Aqueous and Benzene Fractions of Two TNT Wastewaters and a Partially Photolyzed Solution of 2,4,-DNT to Daphnia magna and Fathead Minnows | 32 |
| 7 | Acute Toxicity to Fathead Minnows and Daphnia magna of Organic Compounds Identified in Condensate Wastewater | 34 |
| 8 | Contribution of Selected Individual Organic Components to the Acute Toxicity of Condensate Wastewater | 35 |
| 9 | Acute Toxicity to Daphnia magna of Blend 3 of Condensate Water Irradiated at Different Flow Rates | 37 |
| 10 | Acute Toxicity to Daphnia magna of the Final Blend of Condensate Water Irradiated at Different Flow Rates | 38 |
| 11 | Changes in the Relative Concentration of Compounds in Blend 3 Condensate Water after UV Irradiation at Different Flow Rates | 39 |
| 12 | Changes in the Relative Concentration of Compounds in the Final Blend of Condensate Water after UV Irradiation at Different Flow Rates | 40 |
| 13 | Acute Toxicity of 2,4-DNT and Blend 1 of Condensate Water to Four Species of Fish. | 41 |
| 14 | Acute Toxicity of 2,4-DNT and Blend 1 of Condensate Water to Four Species of Invertebrates. | 42 |

TABLES (continued)


15 Acute Toxicity to Bluegill and Daphnia magna of the Final Blend of Condensate Water before and after Photoirradiation . . . . . 42

16 Effect of 2,4-DNT on Population Growth of S. capricornutum and M. aeruginosa Exposed under Static Conditions for 4 to 14 days . . . . . 44

17 Effect of 2,4-DNT on Population Growth of A. flos-aquae and N. pelliculosa Exposed under Static Conditions for 14 days . . . . . 44

18 Effect of Condensate Water (Blend 1) on Population Growth of Four Species of Algae Exposed under Static Conditions for up to 14 days . . . . . 45

19 Effect of Photolyzed and Non-Photolyzed Condensate Water (Final Blend) on the Growth of S. capricornutum . . . . . 46

20 Effects of Water Temperature, Hardness, and pH on the Acute Toxicity of 2,4-DNT to the Bluegill . . . . . 47

21 Effects of Water Temperature, Hardness, and pH on the Acute Toxicity of Condensate Water (Blend 1) to the Bluegill . . . . . 48

22 Effects of Water Temperature, Hardness, and pH on the Acute Toxicity of Condensate Water (Final Blend) to the Bluegill . . . . . 49

23 Effects of Water Temperature, Hardness, and pH on the Acute Toxicity of Photolyzed Condensate Water (Final Blend) to the Bluegill . . . . . 49

24 Acute Toxicity of Condensate Water and Photolyzed Condensate Water (Blend 2) to Selected Early Life Stages of the Fathead Minnow . . . . . 50

25 Uptake of 2,4-DNT in Condensate Water and 2,4-DNT Alone by Aquatic Organisms, Phase 1, Task 5 . . . . . 52

26 Steady-State BCF Values Calculated from Estimated Log P Values for 30 Condensate Wastewater Compounds . . . . 53

27 Means and Standard Deviations of the Length and Weight of the Control Fish from the Flow-Through Acute Tests on 2,4-DNT and Condensate Water . . . . . 54

TABLES (concluded)


28 Means and Standard Deviation (S.D.) of the Measured *Concentrations of 2,4-DNT Evaluated in the* Flow-Through Acute Tests . . . . . 56

29 Means and Standard Deviations (S.D.) of the Measured Concentrations of Condensate Water Evaluated in the Flow-Through Acute Tests . . . . . 57

30 Summary of Water Quality Measurements Performed During the Flow-Through Acute Tests on 2,4-DNT . . . . . 58

31 Summary of Water Quality Measurements Performed During the Flow-Through Acute Tests on Condensate Water . . . . . 59

32 Acute Toxicity of 2,4-DNT to Selected Species of Fish and Invertebrates Exposed under Flow-Through Conditions . . . 60

33 Acute Toxicity of Condensate Water (Final Blend) to Selected Species of Fish and Invertebrates Exposed under Flow-Through Conditions . . . . . 60

## ILLUSTRATIONS


| | | |
|---|---|---|
| 1 | Capillary Gas Chromatographic Profile of Condensate Components in the Melt . . . . . | 25 |
| 2 | Liquid Chromatographic Profile of Condensate Water . . . . . | 28 |

# INTRODUCTION

This report is the second in a series of four that present and discuss the results of a laboratory investigation on the toxicity to aquatic organisms of wastewater produced during the manufacture and processing of 2,4,6-trinitrotoluene (TNT), an explosive used extensively for military purposes. The investigation, sponsored by the U.S. Army Medical Research and Development Command (USAMRDC) and performed by SRI International, was conducted to develop a data base that could be used by the USAMRDC to assess the probable hazards of TNT production wastewaters to aquatic life.

This volume presents and discusses the results of several studies on condensate wastewater in which short-term exposure tests were used in different ways not only to determine the acute toxicity of the wastewater, but also to determine the acute toxicity of its components and the effects of various environmental factors on the toxicity of the wastewater. The results of these studies were used to determine whether modifications to subsequent parts of the general experimental approach were necessary, the kinds of subchronic and chronic tests needed to complete the evaluation, the kinds of organisms that should be used in the tests, the long-term exposure levels and the water quality conditions that should be maintained during the tests, and whether individual components of the wastewater should be evaluated further.

The studies described in this volume comprise a portion of a comprehensive, four-phased, multitask testing plan. The plan is described in Volume I (Liu et al., 1981), which also presents and discusses the results of similar studies performed on wastewater from facilities engaged in loading, assembling, and packing (LAP) a TNT-based formulation known as Composition B. The equipment, facilities, certain materials, and testing procedures used to evaluate condensate and LAP wastewater were virtually identical and are described in Volume I.

The two other volumes in the series are: Volume III, "Subchronic and Chronic Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene" and Volume IV, "Subchronic and Chronic Toxicity of Condensate Wastewater and 2,4-Dinitrotoluene."

# BACKGROUND

## Source of Condensate Wastewater

The continuous manufacture of the explosive 2,4,6-trinitrotoluene (TNT) at U.S. Army Ammunition Plants (AAPs) produces a wastewater called red water, which is treated by distillation. The distillate is condensed (hence, the term condensate wastewater) and discharged without treatment except for pH adjustment and sedimentation.

Several AAPs are equipped to manufacture TNT. These include the Volunteer plant in Chattanooga, Tennessee; the Radford plant in Radford, Virginia; the Joliet plant in Joliet, Illinois; and the Newport plant in Newport, Indiana.

## Chemical Composition of Condensate Wastewater

Spanggord and coworkers (1978) analyzed numerous samples of condensate wastewater collected over a 12-month period from the Volunteer Army Ammunition Plant (VAAP) and reported his studies in conjunction with those performed under the direction of Dr. N.E. Burlinson of the U.S. Naval Surface Weapons Center. These studies revealed the presence of over 30 organic compounds, all but three of them nitroaromatics. The compounds include toluene, 2,4,6-TNT, 28 nitroaromatic by-products of TNT manufacture, and two compounds whose origins are unclear. These two compounds are N-nitrosomorpholine and N-morpholinoacetonitrile. Both are thought to be derivatives of chemicals added for boiler water treatment at VAAP.

## Toxicity of Condensate Wastewater to Aquatic Organisms

The literature contains no information on the toxicity of condensate wastewater to aquatic organisms. However, some toxicity studies have been performed on some of the components of condensate wastewater and on red water from which condensate wastewater is derived.

According to Degani (1943), red water (red liquor wastes) from a TNT-manufacturing plant killed minnows in 130 to 500 minutes at a concentration of 1750 mg/L total dissolved solids. The same concentration did not kill carp or bullhead catfish. At 7000 mg/L, the effluent killed all three species of fish within 254 minutes.

Burton (1972) reported a 96-hour LC50 of 16 mg/L for an unspecified isomer of dinitrotoluene, which was evaluated with bluegill sunfish.

LeClerc (1960) determined the minimum lethal dose (MLD) of several nitroaromatic compounds using minnows (unspecified species), a six-hour exposure period, and hard and distilled water. The MLDs (in mg/L) are presented below as reported by the author. The isomers of nitrobenzene and dinitrobenzene tested were not reported; mixtures of isomers may have been used.

| Compound | MLD Distilled Water | MLD Hard Water |
|---|---|---|
| Dinitrobenzene | 10 - 12 | 8 - 10 |
| Nitrobenzene | 20 - 4 | 90 - 100 |
| 2-Nitrotoluene | 18 - 20 | 35 - 40 |
| 3-Nitrotoluene | 14 - 18 | 25 - 30 |
| 4-Nitrotoluene | 20 - 2 | 45 - 50 |
| Trinitrotoluene | 4.0 - 5.0 | 4.0 - 5.0 |

From LeClerc (1960)

The literature on the toxicity of toluene is extensive and is only briefly reviewed here. Based on a review of the literature, McKee and Wolf (1963) reported that the lethal concentration of toluene to fish can range from 10 to over 90 mg/L depending on water temperature and the species of fish tested. Wallen and coworkers (1957) tested toluene in turbid water using Gambusia affinis (mosquito fish) and reported a 96-hour LC50 of 1180 mg/L. In tests with Daphnia magna (invertebrate) and Scenedesmus sp. (alga), Bringmann and Kuhn (1959) estimated the lethal threshold concentration of toluene to be 60 mg/L for D. magna and 120 mg/L for the alga. Using hard water, Pickering and Henderson (1966) obtained 96-hour LC50 estimates for toluene ranging from 24.0 to 59.3 mg/L in tests with the fathead minnow, bluegill sunfish, goldfish, and guppy. Using the fathead minnow to determine the effect of water hardness on the acute toxicity of toluene, these researchers obtained 96-hour LC50s of 35.0 mg/L with soft water and 42.3 mg/L with hard water.

Berry and Brammer (1977) reported that the LD50 and nonlethal dose of toluene to 4th instar mosquito larvae (Aedes aegypti) were 21.25 ppm and 9.95 ppm, respectively, for a 24-hour exposure. Bakke and Skjoldal (1979) found that marine isopods (Cirolana borealis) exposed to 5.7 and 125 ppm of toluene died after 400 and 3 hours of exposure, respectively. Korn et al. (1979) eval-

uated the response of pink salmon (Oncorhynchus gorbuscha) and shrimp (Evalus sp.) to toluene at temperatures of 4 - 12°C. They obtained 96-hour TLm estimates of 6.41 - 8.09 and 14.7 - 21.4 mg/L for the salmon and shrimp, respectively.

Schott and Worthley (1974) investigated the toxicity of 2-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-dinitrotoluene, and 2,4,6-trinitrotoluene to duckweed (Lemna perpusilla) at two different pH levels. Effect and no-effect concentrations after 11 days of exposure at pH 6.3 were: 2-nitrotoluene, 100.0 and 10.0 ppm; 4-amino-2-nitrotoluene, 50.0 and 10.0 ppm; 2,4-dinitrotoluene, 0.1 and 0.01 ppm; and trinitrotoluene, 1.0 and 0.5 ppm. Similar concentrations after 11 days of exposure at pH 8.5 were reported as: 2-nitrotoluene, no effect at 100.0 ppm; 4-amino-2-nitrotoluene, 10.0 and 1.0 ppm; 2,4-dinitrotoluene, 1.0 and 0.1 ppm; and trinitrotoluene, 1.0 and 0.1 ppm. The authors felt that the relatively high toxicity of these materials suggested that indiscriminate disposal of TNT wastes and byproducts could be a potentially serious pollution hazard.

Ohmori et al. (1975) investigated the metabolism of 4-nitrotoluene in isolated rat and eel liver homogenates. They found that the eel liver homogenates metabolized the methyl group of the nitrotoluene more slowly than did the rat liver homogenate. They indicated that this observation is consistent with the greater accumulation of toluene in fish compared with mammals.

Wentsel et al. (1979) reviewed the available toxicity data on 1,3-dinitrobenzene and 1,3,5-trinitrobenzene. From the aquatic toxicity data on the former, the authors concluded that the compound is moderately toxic (LC50 estimates between 7 and 10 mg/L) to fish under acute conditions and that invertebrates are less sensitive. Because the material is relatively stable in the environment, they recommended that chronic bioassays be performed. Based on a bioconcentration factor of 10 estimated from log P values, the authors felt that 1,3-dinitrobenzene would not accumulate appreciably in fish. They also felt that the limited acute LC50 data available on 1,3,5-trinitrobenzene (1.0 and 2.7 mg/L, respectively, for fathead minnows and D. magna) indicated that this compound is moderately toxic to fish and invertebrates. In addition, calculated BCF values indicated little potential for bioaccumulation. Because of the slow degradation rate of this material in the environment and the fact that it is a decomposition product of TNT, the authors recommended that chronic bioassays be performed.

Bringmann and Kuhn (1980) evaluated the response of bacteria (Pseudomonas putida), algae (Scenedesmus quadricauda) and a protozoan (Entosiphon sulcatum) to 1,3-dinitrobenzene. Based on inhibition of all multiplication, they found 14 mg/L to be the toxic threshold for the bacteria exposed for 16 hours, 0.7 mg/L for algae exposed for 7 days, and 0.76 mg/L for protozoa exposed for 72 hours.

Some condensate water components appear to affect the taste of fish. In a study to determine the cause of the unpleasant taste of fish caught in streams

contaminated by wastes from an explosive manufacturing plant, Bandt (1946) discovered that the tainting was caused primarily by the presence of mononitrotoluenes, nitrobenzenes, and orthochlorobenzenes in the water.

Field Studies

Sullivan and coworkers (1977a, 1977b) surveyed the biological communities at various locations in Chickmauga Lake on the Tennessee River where TNT manufacturing wastewater is discharged from the Volunteer Army Ammunition Plant in Chattanooga, Tennessee. The surveys were performed in June, August, and December of 1975 and in March of 1976. Of particular interest were the biological communities in Waconda Bay, where the wastewater outfall is located.

During the June and August surveys, they found up to 345μg/L of 2,4- and 2,6-dinitrotoluene and 2,4,6-trinitrotoluene in the water samples collected near the outfall. The concentrations of these compounds decreased to below detectable limits at a distance of 0.5 to 0.75 miles downbay of the outfall. The authors reported that whenever the total concentration of these three compounds exceeded 20μg/L, effects were observed in the periphyton and macroinvertebrate communities. It was not determined, however, whether the observed effects were caused by these compounds or other components of the wastewater.

During the surveys performed in December 1975 and March 1976, Sullivan and coworkers (1977b) determined the concentrations in water and sediment samples of the same compounds measured during the earlier surveys plus 1,3-dinitrobenzene and 1,3,5-trinitrobenzene. In December, the average total concentration of these compounds in the water samples ranged up to 397 μg/L, with 2,4-dinitrotoluene and 2,4,6-trinitrotoluene accounting for about 75 percent of the total. In March, the maximum average was 102 μg/L, with 2,6-dinitrotoluene and 2,4,6-trinitrotoluene predominating. The highest concentrations were found near the outfall. Interestingly, almost no 1,3,5-trinitrobenzene was found in the wastewater itself; however, water samples from Waconda Bay contained up to about 21 μg/L of this compound, and sediment samples contained up to 304 μg/L.

This report was unclear; however, it appears that no effects attributable to the effluent were observed on the periphyton or macroinvertebrate populations colonizing natural and artificial substrates at stations where the total concentration of the five compounds was less than 25μg/L.

At the Joliet Army Ammunition Plant, up to 210μg/L of 2,6-dinitrotoluene, 1710 μg/L of 2,4-dinitrotoluene, and 1140 μg/L of 2,4,6-trinitrotoluene were found in water samples from Grant Creek, which receives TNT manufacturing wastewater from the plant. The standing crops and species diversity of the macroinvertebrates and periphyton in the creek were markedly reduced in areas where the total concentration of these compounds exceeded 50μg/L; however, that the observed effects were actually caused by these compounds was not determined (Dr. W.H. van der Schalie, USAMRDC, personal communication).

## EXPERIMENTAL APPROACH

The general testing plan is described in detail in Volume I (Liu et al., 1981). This section only identifies the tasks related to a toxicity evaluation of condensate wastewater and 2,4-DNT and the objectives of the tasks for each phase.

Phase I: Preliminary Studies

Task 1. Objective: To determine the effect of photoirradiation of red water, condensate wastewater, and 2,4-DNT on their acute toxicity and thereby determine the extent to which the photoirradiated materials should be studied.

Task 2. Objective: Partially identify the most toxic components of red water, condensate wastewater, and photolyzed 2,4-DNT by determining the acute toxicity of their aqueous and benzene fractions.

Task 3. Objective: To determine the acute toxicity of selected organic components of condensate wastewater to identify those that should be used to prepare synthetic condensate wastewater (condensate water) and those that should be studied more extensively.

Phase II: Acute Toxicity Studies on Condensate Water and Additional Studies on 2,4-DNT

Task 1. Objective: To establish an irradiation endpoint for preparing photolyzed condensate water so that its toxicity would be consistent from batch to batch.

Task 2. Objective: To determine the relative sensitivity of 12 aquatic species from three trophic levels to condensate water and 2,4-DNT.

Task 3. Objective: To determine the effect of water quality on the acute toxicity of condensate water and 2,4-DNT.

Task 4. Objective: To determine the acute toxicity of condensate water to selected early life stages of the fathead minnow.

Task 5. Objective: To obtain a rough estimate of the propensity of 2,4-DNT to bioconcentrate in aquatic organisms and thus determine the need to conduct full-scale bioconcentration tests.

Phase III: Definitive Acute Toxicity Studies

Task 1. Objective: To determine the incipient LC50 of condensate water and 2,4-DNT using selected aquatic species.

## MATERIALS AND EQUIPMENT

### Materials

#### Materials Evaluated

The materials evaluated for toxicity were condensate wastewater, red water, condensate water (synthetic condensate wastewater), and 32 organic components of condensate wastewater. Table 1 lists the materials and their sources. Fourteen of the organic compounds were synthesized at SRI International. The methods used to synthesize and analyze them have been described by Spanggord and coworkers (1978). All of the compounds were determined to be at least 95 percent pure. N-nitrosomorpholine and N-morpholinoacetonitrile were not tested because they are not direct products or by-products of TNT manufacture.

The bioconcentration studies were performed with uniformly ring-labelled $^{14}$C-2,4-DNT, which was manufactured by the New England Nuclear Corporation. Its specific activity and lot number were 2.52 $\mu$Ci/mM and 879-171, respectively.

Authentic, untreated red water and condensate wastewater were obtained from the VAAP, Chattanooga, Tennessee, and were shipped to SRI International in polyethylene-lined 55-gallon steel drums, where they were stored at 4°C for several months until used.

During the study, we developed and tested four condensate-water blends. Table 2 shows the composition of each blend. Each compound was chosen for inclusion in a blend if it: (1) was found in measurable quantities in at least 10 percent of the 79 condensate wastewater samples analyzed, (2) exhibited mutagenicity in the Ames bacterial mutagenicity test, or (3) had a 48-hour LC50 in Daphnia magna or a 96-hour LC50 in fathead minnows of 1.0 mg/L or less. Data pertinent to the third criterion were developed in this study. Data pertaining to the other criteria were developed by other investigators and summarized by Pearson and coworkers (1979). The method used to establish the concentration of the compounds in the blends is described by Spanggord and coworkers (1978) and Pearson and coworkers (1979). The blends were developed as the inclusion criteria data were developed.

#### Test Organisms

Twelve species of aquatic organisms were used in the study. They were:

Algae

Selenastrum capricornutum (green alga)
Microcystis aeruginosa (bluegreen alga)
Anabaena flos-aquae (bluegreen alga)
Navicula pelliculosa (diatom)

Invertebrates

Daphnia magna (cladoceran)
Hyalella azteca (amphipod)
Tanytarsus dissimilis (midge)
Lumbriculus variegatus (oligochaete)

Fish

Pimephales promelas (fathead minnow)
Lepomis machrochirus (bluegill sunfish)
Ictalurus punctatus (channel catfish)
Salmo gairdnerii (rainbow trout)

The algal assays were initiated with cells obtained from stock cultures in the growth phase. Tests with D. magna were performed on first instars (less than 12 hours old), and tests with T. dissimilis were performed on mixed lots of instar stages 1 to 3 (up to 24-hours post-hatch). In the tests with H. azteca and L. variegatus, we used individuals of unknown age. The fish used in the tests were juveniles; however, the ages of the bluegills, catfish, and trout were not known. The minnows were approximately 90 days old.

Volume I identifies the sources of these organisms and describes the methods we used to culture, rear, and maintain them, as well as the acclimation and other pretest procedures.

Diluent Water

Dechlorinated tap water was used to prepare the stock solutions of condensate water and the 32 organic compounds, and to dilute all of the test substances to the desired concentrations for testing with the animal species. In the tests with algae, however, we used an EPA-recommended algal nutrient medium (EPA, 1971). Dechlorinated tap water was also used to culture, rear, and maintain all of the animal species.

The physical and chemical characteristics of the dechlorinated water, its sources, and the methods used to dechlorinate and treat it are described in Volume I.

Equipment

Volume I describes the equipment used in this study.

Table 1. NAME AND SOURCE OF THE MATERIALS TESTED

| Toxicant | Source |
|---|---|
| Red water | Volunteer Army Ammunition Plant (VAAP) |
| Condensate wastewater | VAAP |
| Synthetic condensate wastewater | Prepared at SRI |
| Toluene | Mallinckrodt, Lot No. BPB |
| 2-Nitrotoluene | Eastman Organic Chemicals, Lot No. not given |
| 3-Nitrotoluene | Unknown |
| 4-Nitrotoluene | Matheson Company, Lot No. 303117 |
| 3-Nitrobenzonitrile | Aldrich Chemical Co., Lot No. 090357CB |
| 4-Nitrobenzonitrile | Aldrich Chemical Co., Lot No. not given |
| 2-Amino-4-nitrotoluene | Pfaltz and Bauer, Lot No. not given |
| 2-Amino-6-nitrotoluene | Aldrich Chemical Co., Lot No. 031207 |
| 3-Amino-4-nitrotoluene | Synthesized at SRI |
| 4-Amino-2-nitrotoluene | Unknown |
| 3-Methyl-2-nitrophenol | Aldrich Chemical Co., Lot No. 011454 |
| 5-Methyl-2-nitrophenol | Aldrich Chemical Co., Lot No. 122929 |
| 1,3-Dinitrobenzene | Eastman Organic Chemicals, Lot No. 601-344 |
| 2,3-Dinitrotoluene | Synthesized at SRI |
| 2,4-Dinitrotoluene | ICN, Lot No. 54823 |
| 2,5-Dinitrotoluene | Synthesized at SRI |
| 2,6-Dinitrotoluene | Aldrich Chemical Co., Lot No. 031947 |
| 3,4-Dinitrotoluene | Aldrich Chemical Co., Lot No. AB082467 |
| 3,5-Dinitrotoluene | Synthesized at SRI |
| 3,5-Dinitroaniline | Aldrich Chemical Co., Lot No. 011477AB |
| 1,5-Dimethyl-2,4-dinitrobenzene | Synthesized at SRI |
| 2-Amino-3,6-dinitrotoluene | Synthesized at SRI |
| 2-Amino-4,6-dinitrotoluene | Naval Surface Weapons Center |
| 3-Amino-2,4-dinitrotoluene | Synthesized at SRI |
| 3-Amino-2,6-dinitrotoluene | Synthesized at SRI |
| 4-Amino-2,6-dinitrotoluene | Naval Surface Weapons Center |
| 4-Amino-3,5-dinitrotoluene | Synthesized at SRI |
| 5-Amino-2,4-dinitrotoluene | Synthesized at SRI |
| 2,4-Dinitro-5-methylphenol | Synthesized at SRI |
| 1,3,5-Trinitrobenzene | Synthesized at SRI |
| 2,3,6-Trinitrotoluene | Synthesized at SRI |
| 2,4,6-Trinitrotoluene | E.I. Dupont NeMours, Lot No. not given |

Table 2. COMPOSITION OF THREE INTERIM BLENDS AND THE FINAL BLEND OF CONDENSATE WATER

| Compound | Relative Concentration (%) | | | |
|---|---|---|---|---|
| | Blend 1 | Blend 2 | Blend 3 | Final Blend |
| Toluene | 0.59 | 0.59 | 0.59 | 0.590 |
| 2-Nitrotoluene | 0.075 | 0.09 | 0.089 | 0.089 |
| 4-Nitrotoluene | 0.28 | 0.30 | 0.30 | 0.295 |
| 3-Nitrobenzonitrile | -- | -- | 0.10 | 0.035 |
| 4-Nitrobenzonitrile | -- | -- | 0.10 | 0.027 |
| 2-Amino-4-nitrotoluene | -- | 0.03 | 0.097 | 0.097 |
| 2-Amino-6-nitrotoluene | -- | -- | 0.03 | 0.030 |
| 3-Amino-4-nitrotoluene | -- | -- | 0.097 | 0.080 |
| 3-Methyl-2-nitrophenol | 0.03 | 0.03 | 0.03 | 0.035 |
| 5-Methyl-2-nitrophenol | -- | 0.09 | 0.06 | 0.094 |
| 1,3-Dinitrobenzene | 13.58 | 12.03 | 12.01 | 11.803 |
| 2,3-Dinitrotoluene | 1.52 | 1.20 | 1.18 | 1.180 |
| 2,4-Dinitrotoluene | 50.72 | 44.20 | 44.10 | 43.377 |
| 2,5-Dinitrotoluene | 1.22 | 1.20 | 1.18 | 1.180 |
| 2,6-Dinitrotoluene | 22.01 | 21.95 | 21.90 | 21.541 |
| 3,4-Dinitrotoluene | 1.51 | 1.56 | 1.475 | 1.475 |
| 3,5-Dinitrotoluene | 1.51 | 1.56 | 1.53 | 1.534 |
| 3,5-Dinitroaniline | -- | -- | 0.01 | 0.171 |
| 1,5-Dimethyl-2,4-dinitrobenzene | 1.50 | 1.20 | 1.29 | 1.151 |
| 2-Amino-3,6-dinitrotoluene | -- | -- | 0.089 | 0.089 |
| 2-Amino-4,6-dinitrotoluene | 0.50 | 0.06 | 0.059 | 0.059 |
| 3-Amino-2,4-dinitrotoluene | -- | 4.52 | 4.50 | 4.426 |
| 3-Amino-2,6-dinitrotoluene | -- | 3.61 | 3.60 | 3.541 |
| 4-Amino-2,6-dinitrotoluene | 1.83 | 1.80 | 1.77 | 1.770 |
| 4-Amino-3,5-dinitrotoluene | 0.60 | 0.60 | 0.59 | 0.590 |
| 5-Amino-2,4-dinitrotoluene | 2.45 | 2.11 | 2.07 | 2.066 |
| 2,4-Dinitro-5-methylphenol | -- | -- | 0.14 | 0.251 |
| 1,3,5-Trinitrobenzene | -- | 0.02 | 0.02 | 0.451 |
| 2,3,6-Trinitrotoluene | -- | -- | 0.06 | 0.791 |
| 2,4,6-Trinitrotoluene | 1.50 | 1.20 | 1.18 | 1.180 |

# METHODS

## Acute Toxicity Determination

The manual entitled "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (EPA, 1975) was used as a guide for designing and conducting the acute toxicity tests with the animal species. Static techniques were used in Phases I and II and flow-through techniques were used in Phase III. Acceptable levels of mortality in the controls were 10 percent for fish tests and 20 percent for invertebrate tests. Instances where these levels were exceeded are noted in the text. The tests with algae were performed using procedures described in the manual "Algal Assay Procedures: Bottle Test" (EPA, 1971). Volume I describes the testing methods in detail.

## Bioconcentration

The bioconcentration tests performed were intended only to obtain information that would enable us to decide if full-scale bioconcentration tests should be performed on 2,4-DNT. For this reason, the test organisms were exposed to $^{14}$C-2,4-DNT for only four days.

In one series of tests, bluegills (12 juveniles/30 L of 2,4-DNT solution), D. magna (100 adults/2 L), L. variegatus (50 adults/2 L), and S. capricornutum (initially about 10,000 cells/0.1 L) were exposed to solutions containing 1 mg/L of $^{14}$C-2,4-DNT. In another series of tests, the same kinds of organisms were exposed to about 3 mg/L of Blend 3 of condensate water to which approximately 300 dpm of $^{14}$C-2,4-DNT were added per milliliter. The amounts of radioactivity in the test medium were determined at the beginning of the tests and in the whole bodies or cells of the invertebrates and algae after the four-day exposure period. However, with the bluegills, radioactivity measurements were performed on samples of the viscera (gills and kidneys excluded) and dorsolateral muscles on the second and fourth days of exposure, and 3 and 10 days after the exposed fish had been transferred to clean flowing water. At each sampling period, the tissues from three fish were analyzed. Each group of three fish was exposed in a separate container. The radioanalytical procedures are described in Volume I.

## Preparation of Condensate Water

The four condensate water blends were prepared in the same manner. Each blend was prepared so that the concentrations of the compounds totalled about 150 mg/L. To prepare the blends, measured volumes of dechlorinated tap water and acetone stock solution of the compounds were combined in plastic-lined 55-gallon drums, the mixture was stirred for 48 hours with a 0.33-hp electric stirrer, and the mixture was recirculated for four hours through a 5-$\mu$m cartridge filter.

The acetone stock solution of the solid ingredients (only toluene and 2-nitrotoluene were not solids) was prepared in several steps:

1. Each compound was weighed to within 1 percent of the desired weight (based on the relative percentages shown in Table 1) using

an analytical balance or a top-loading balance for those compounds whose desired weight exceeded the capacity of the analytical balance.

2. After the compounds were weighed, they were transferred to a 2000-ml Pyrex glass Erlenmeyer flask using a Pyrex glass funnel and a camel hair brush. The flask was partially submerged in an oil bath, which was slowly heated to 80°C and maintained at 80°C for 45 minutes. At this temperature, the compound mixture was stirred frequently. The temperature of the bath was increased to 110°C and kept there while stirring continued. When the mixture appeared as a dark brown, glassy, homogeneous liquid, devoid of crystalline material (20 minutes), it was removed from the bath and allowed to cool for two hours at room temperature. After the mixture cooled, toluene and 2-nitrotoluene were added to it by volumetric pipette, the mixture was returned to the oil bath, and the temperature was increased to 120°C over 45 minutes. During this period, the mixture was stirred frequently. This mixture was called the melt.

3. The hot mixture was poured directly into a Pyrex glass beaker partially submerged in a slurry of dry ice and acetone, and allowed to freeze for two to three hours. The frozen mixture was then covered and kept in a refrigerator (about 4°C) for 24 to 48 hours. After this period, the mixture was thawed at room temperature, thoroughly stirred, and returned to the refrigerator until needed.

4. Whenever condensate water was needed, the mixture was thawed at room temperature and stirred thoroughly, and then a weighed portion of it was dissolved in reagent grade acetone.

Photoirradiation Methods

To prepare photolyzed solutions of 2,4-DNT and photolyzed condensate wastewater, condensate water, and red water, the pH of these materials was adjusted to 7 using 5N NaOH or 0.1N HCl and they were exposed to filtered ultraviolet light (simulated sunlight) in the photolytic reactors described in Volume I. The photolyzed materials used in Phase I of the study were batch irradiated; whereas the photolyzed materials used in Phases II and III were irradiated in a flow-through reactor. To determine the degree of photolysis, samples of the materials were periodically analyzed for an indicator compound as they were being irradiated. For red water, the indicator compound was 2,4,6-TNT. For the other materials, it was 2,4-DNT.

Preparation of the Aqueous and Benzene Fractions

About six liters each of condensate wastewater, red water, and a solution of partially photolyzed 2,4-DNT were shaken for three minutes in separate separatory funnels with an equal volume of benzene, then allowed to stand until the aqueous and benzene fractions separated. The aqueous fractions were drawn off, and benzene residues in those fractions removed by heating the fraction to 60°C under vacuum and aerating it with nitrogen gas. Samples

of the aqueous fractions were analyzed periodically by gas chromatography for benzene.

After all traces of benzene were removed from the aqueous fractions, 50 ml of the aqueous and benzene fractions were lyophilized, and the dry residues weighed to determine the concentration of total dissolved solids (TDS) in each fraction. Another 20-ml aliquot was removed from each fraction and analyzed for 2,4-DNT or 2,4,6-TNT (red water only).

To prepare the samples of the benzene fractions for 2,4-DNT or 2,4,6-TNT analysis, m-dinitrobenzene (internal standard) was added to the samples, the samples were rotary evaporated to dryness, and the dry residue was dissolved in 1 ml of dichloromethane. The aqueous samples were prepared by extracting them with equal volumes of ether, filtering the ether extracts through magnesium sulfate, adding m-dinitrobenzene to the filtrates, rotary evaporating the resulting mixtures to dryness, and dissolving the dry residues in 1 ml of dichloromethane. Each residue-dichloromethane solution was analyzed for 2,4-DNT or 2,4,6-TNT by gas chromatography.

## Analytical Chemistry

### General

During the study, the concentrations of 2,4,6-TNT in red water and 2,4-DNT in condensate wastewater were determined. The melt used to prepare the various condensate water blends, the blends themselves, and the aqueous stock solutions of each of the compounds tested were also analyzed. Two analytical techniques were used--gas chromatography (GC) and high-pressure liquid chromatography (HPLC).

### Preparation of Analytical Standards

Standards were prepared by dissolving a known weight of the standard compound in reagent grade ethyl acetate in an appropriately sized glass volumetric flask. The flasks were wrapped in aluminum foil to prevent photodecomposition. Weights were determined with a Cahn electrobalance.

### GC Analysis of the Melt

The melt was analyzed with a gas chromatograph equipped with a capillary column under the following conditions:

Instrument: Varian 2700 (modified for capillary use)

Column: 40-meter glass capillary coated with OV101 (liquid phase)

Temperature program: 116°C-220°C at 4°C/min with 15-min hold

Detector: Flame ionization

Detector Temperature: 250°C

Injector Temperature: 250°C

Carrier Gas: $N_2$

Air Flow: 200 ml/min

$H_2$ Flow: 30 ml/min

Make-up Gas: 30 ml/min

Split Ratio: 190/1

Range: $10^{-11}$ amp/mv

Injection Volume: 2-3 $\mu$ L

To identify the retention times of all compounds in the melt, a standard solution of eight compounds with well-known retention times was prepared then analyzed repeatedly, one or two more compounds added each time and the retention times of the new peaks duly noted.

Before a melt of a blend was analyzed, a standard solution containing the components of the melt or blend was checked for response. Injections were made until two consecutive injections produced similar response patterns. A separate standard solution containing 3-methyl-2-nitrophenol and 5-methyl-2-nitrophenol was analyzed similarly, but as their trimethyl-silyl derivatives.

A known amount of the condensate melt (about 800 to 1000 mg) was diluted to 10 ml with reagent grade ethyl acetate, and one ml of the solution was added to one ml of the internal standard (1,4-dinitrobenzene). A 2 ml sample of this mixture was injected into the gas chromatograph. To quantitate compounds with relatively low concentrations, we combined one ml of the ethyl acetate solution with 1 ml of diluted internal standard, concentrated the mixture to 0.3 to 0.5 ml, and injected 3 to 4 ml into the gas chromatograph.

A typical chromatogram of a condensate melt is presented in Figure 1. Table 3 presents a summary of condensate component response factors (to 1,4-dinitrobenzene) and a comparison of observed and expected percentage distributions.

Analysis of Condensate Water

In the acute toxicity studies, condensate water was analyzed by reverse-phase HPLC; this technique was used to determine the total concentration of the components in the samples rather than the concentration of each component. Capillary GC was used in the subchronic and chronic toxicity studies (Volume IV).

Standard solutions were prepared by dissolving a known amount of condensate melt in HPLC grade methanol (150:200 mg/L). Volumetric dilutions from this standard were made for lower concentration standards.

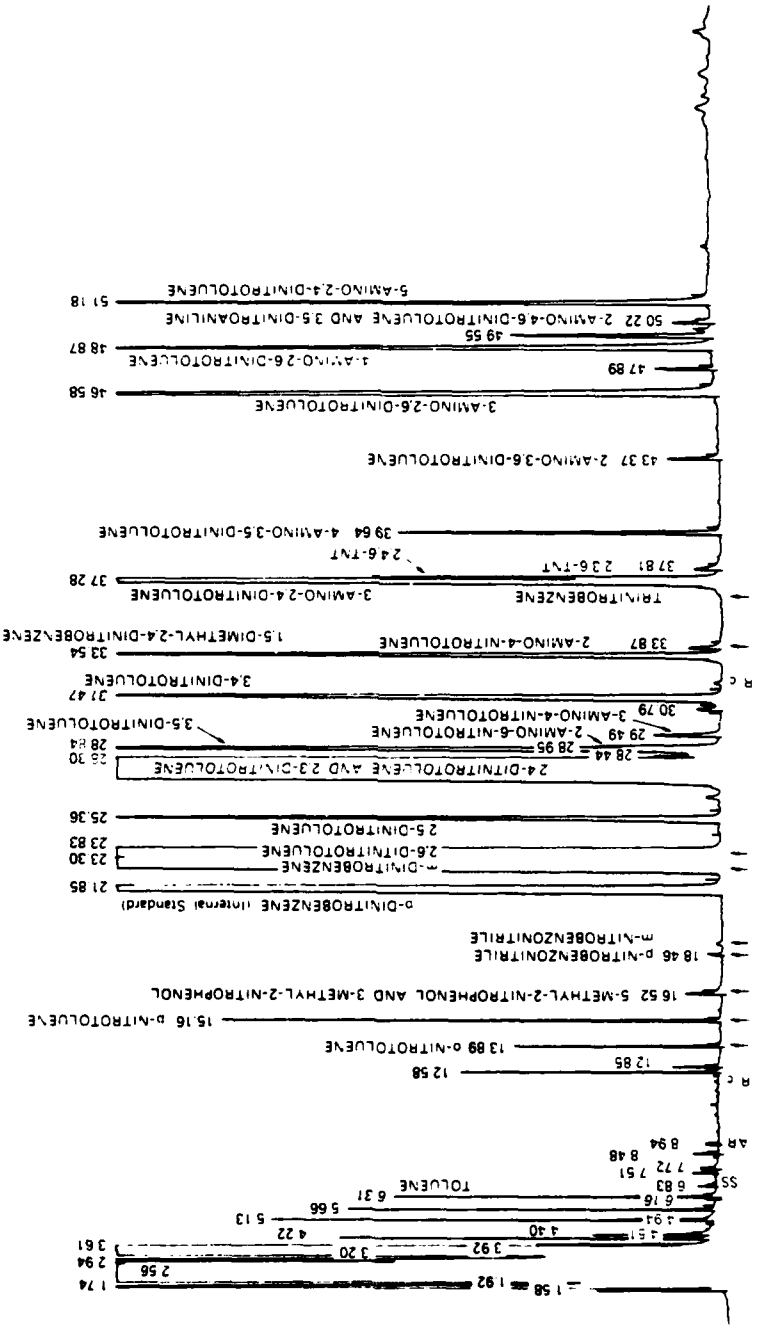


FIGURE 1 CAPILLARY GAS CHROMATOGRAPHIC PROFILE OF CONDENSATE COMPONENTS IN THE MELT

Table 3. CONDENSATE MELT ANALYSIS DATA SUMMARY (for melt produced 3/79)

Total Concentration of Melt 91.7 mg/ml

| Compound | $1/R_f$ | Area Compound Peak | Area I.S.[b] Peak | Conc. I.S.[b] (mg/ml) | Relative Percentage Observed | | Relative Percentage Expected |
|---|---|---|---|---|---|---|---|
| 2-NT[c] | 0.638 | 281<br>1757 | 18416<br>33332 | 10.36<br>3.45 | 0.110<br>0.116 | 0.113 | 0.089 |
| 4-NT | 0.639 | 775<br>4788 | 18416<br>33332 | 10.36<br>3.45 | 0.304<br>0.320 | 0.312 | 0.295 |
| 1,3-DNB[d] | 0.979 | 18138<br>16476 | 18416<br>15774 | 10.36<br>10.36 | 10.89<br>11.55 | 11.22 | 11.80 |
| 2,6-DNT[e] | 0.865 | 41435<br>37839 | 18416<br>15774 | 10.36<br>10.36 | 21.98<br>23.44 | 22.71 | 21.54 |
| 2,5-DNT | 0.896 | 2054<br>1858 | 18416<br>15774 | 10.36<br>10.36 | 1.13<br>1.19 | 1.16 | 1.18 |
| 3,5-DNT | 0.958 | 2587<br>2296 | 18416<br>15774 | 10.36<br>10.36 | 1.52<br>1.56 | 1.55 | 1.53 |
| 2,4-DNT[a] | 0.889 | 81887<br>73744 | 18416<br>15774 | 10.36<br>10.36 | 43.48<br>45.77 | 44.83 | 43.37 |
| 3,4-DNT | 0.899 | 2603<br>2327 | 18416<br>15774 | 10.36<br>10.36 | 1.44<br>1.50 | 1.47 | 1.48 |
| 1,5-DM-DNB[f] | 0.934 | 2282 | 18416 | 10.36 | 1.22 | 1.27 | |
| 2,4-DNT | | 2099 | 15744 | 10.36 | 1.31 | | |
| 1,3,5-TNB[g] | 1.61 | 1952 | 33332 | 3.45 | 0.35 | | 0.45 |
| 3-A-2,4-DNT | 1.08 | 6055<br>5988 | 18416<br>15774 | 10.36<br>10.36 | 4.41<br>4.63 | 4.52 | 4.43 |
| 4-A-3,5-DNT | 1.22 | 676<br>3974 | 18416<br>33332 | 10.36<br>3.45 | 0.50<br>0.55 | 0.53 | 0.59 |
| 2,3,6-TNT | 4.41 | 685 | 3332 | 3.45 | 0.34 | | 0.79 |
| 3-A-2,6-DNT | 1.14 | 4743<br>4544 | 18416<br>15774 | 10.36<br>10.36 | 3.31<br>3.71 | 3.51 | 3.54 |
| 2-A-3,6-DNT | 1.12 | 634 | 33332 | 3.45 | 0.08 | | 0.09 |
| 4-A-2,6-DNT | 1.17 | 2175<br>2130 | 18416<br>15774 | 10.36<br>10.36 | 1.56<br>1.78 | 1.67 | 1.77 |
| 3,5-DNA[h] | 1.05 | 2975 | 33332 | 3.45 | 0.35 | | 0.17 |
| 5-A-2,4-DNT | 1.23 | 14844<br>2130 | 33332<br>15744 | 3.45<br>10.36 | 2.06<br>1.88 | 1.97 | 2.07 |

[a] Peak areas given include another unresolved peak. In each case the expected percentages of these are subtracted to obtain percentages given ($1/R_f$ values are similar for both pairs).

[b] I.S. denotes internal standard.

[c] Nitrotoluene.

[d] Dinitrobenzene.

[e] Dinitrotoluene.

[f] Dimethyl-2,4-DNB.

[g] Trinitrobenzene.

[h] Dinitroaniline.

One $\mu$L of the internal standard was combined with 1 to 3 ml of sample and injected on the chromatograph. Injection volumes of 10 to 400 $\mu$L were made depending on the concentration of the sample. Because the total concentration was desired, resolution of each compound is not required. A convenient elution time (200 to 300 seconds) produced 3 to 5 major peaks. The total peak area was determined and the total concentration of the condensate components calculated.

The conditions used to analyze the condensate water samples are as follows:

Instruments: Spectra Physics Model 3500 liquid chromatograph

Spectra Physics Autolab minigrator integrator

UV absorbance detector

Omniscribe recorder

System automated January 1980 with addition of Waters Association WISP 710A autosampler, and Waters Radial Compression Module RCM-100

Column: $\mu$-Bondapak $C_{18}$ (3.2 mm I.D. x 25 cm), Waters Radial Pak A or equivalent[1]
Mobile Phase: methanol/$H_2O$ (65/35)
Flow Rate: 2.6 mL/min[2]
Injection Volume: 10-400 $\mu$L
Detection: UV absorbance at 254 nm
Range, 0.02-0.08

A typical chromatographic profile for a condensate water sample appears in Figure 2.

Analysis of Individual Compounds

All of the individual compounds were analyzed by HPLC under the following conditions:

Column: 4mm X 30cm $\mu$ Bondapak $C_{18}$ (Waters Assoc.)
Solvent: 50% $H_2O$/50% $CH_3OH$
Flow Rate: 1.6 mL/min[2]
Detection: UV at 254 nm

Details of the analyses have been reported by Spanggord and coworkers (1978).

Statistical Methods

To compute the 50 percent lethal or effective concentrations (LC50) of the test materials and the 95 percent confidence limits of these estimated concentrations, we used either the probit or the binomial methods. The presence

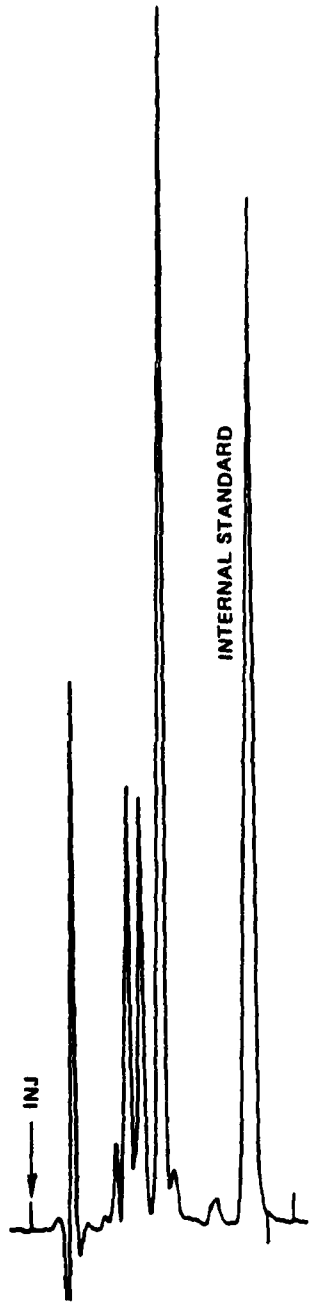


FIGURE 2 LIQUID CHROMATOGRAPHIC PROFILE OF CONDENSATE WATER

of statistically significant differences between LC50s was determined by examining the 95 percent confidence limits of the two statistics and applying Student's t Test when necessary. The control data from the algal assays were treated to an outlier test; then to the F test. More detailed descriptions of these statistical methods are presented in Volume I.

## RESULTS AND DISCUSSION

### Phase I: Preliminary Studies

#### Task 1: Effect of Photoirradiation on the Acute Toxicity of 2,4-DNT, Red Water, and Condensate Wastewater

Table 4 shows the concentrations of 2,4-DNT in undiluted condensate wastewater and the stock solution of 2,4-DNT and the concentrations of 2,4,6-TNT in undiluted red water before and after photoirradiation.

Table 4. CONCENTRATIONS OF 2,4-DNT OR 2,4,6-TNT IN SAMPLES OF RED WATER, CONDENSATE WASTEWATER, AND THE STOCK SOLUTION OF 2,4-DNT BEFORE AND AFTER PHOTOIRRADIATION

| Test Material | Compound Analyzed | Concentration of Compound (mg/L) Non-photolyzed | 50% photolyzed | 100% photolyzed |
|---|---|---|---|---|
| Red Water | 2,4,6-TNT | 4 | 2 (50)[a] | <0.05 (100) |
| Condensate | 2,4-DNT | 62 | 32 (52) | <0.05 (100) |
| 2,4-DNT | 2,4-DNT | 150 | 75 (50) | <0.05 (100) |

[a]Values in parentheses denote the actual percentage of photolysis.

Table 5 presents the 96-hour LC50s of the nonphotolyzed and of the 50 and 100 percent photolyzed samples. The LC50s of the wastewater samples are expressed in mg/L total dissolved solids (TDS); the LC50s of the 2,4-DNT solutions are expressed in mg/L 2,4-DNT. All of the LC50s were computed using the TDS or 2,4-DNT concentrations in the nonphotolyzed samples so that the effects of irradiation, if any, might be revealed.

Table 5. ACUTE TOXICITY TO FATHEAD MINNOWS OF PHOTOLYZED AND NON-PHOTOLYZED SAMPLES OF RED WATER, CONDENSATE WASTEWATER, AND 2,4-DNT

| Test Material | Degree of Photolysis (%)[d] | 96-hour LC50 and 95% Confidence Limits (mg/L) |
|---|---|---|
| Red Water[a] | 0 | 360 (291-765)[b] |
| | 50 | 199 (122-765) |
| | 100 | 398 (336-765) |
| Condensate[b] | 0 | 185 (164-207) |
| | 52 | 214 (188-241) |
| | 100 | 373 (348-413) |
| 2,4-DNT | 0 | 31.4 (29.4-34.0) |
| | 50 | >35.0 (N.C.)[c] |
| | 100 | 64.8 (56.0-75.0) |

[a]Concentrations in Appendix are expressed in percentage of stock solution. See Table 6 for total dissolved solids concentration of the stock solutions.

[b]Test solutions aerated.

[c]Not calculated.

[d]Based on analysis of single compounds; see Table 4.

The 96-hour LC50 for 50 percent photolyzed red water was lower than those computed for the nonphotolyzed and 100 percent photolyzed red water samples; however, none of the LC50s appear different from each other. It was concluded from the data that exposure to simulated sunlight has no appreciable effect on the acute toxicity of red water.

The 96-hour LC50s of the condensate wastewater and 2,4-DNT samples increased as the degree of photolysis of the 2,4-DNT components in the samples increased, indicating that exposure to simulated sunlight reduced the toxicity of condensate wastewater and 2,4-DNT. Reducing the 2,4-DNT component by about 50 percent did not significantly affect the toxicity of condensate wastewater. The 96-hour LC50 of the 50 percent photolyzed sample of 2,4-DNT was higher than the highest tested concentration; hence the degree to which partial photolysis affected its toxicity could not be determined.

Complete photolysis of the 2,4-DNT solution and of 2,4-DNT in condensate wastewater reduced the toxicity of the solution and wastewater by about 50 percent. This reduction was statistically significant ($p \leq 0.05$). It thus appears that the phototransformation products of 2,4-DNT and other photolabile compounds in condensate wastewater are either less toxic than their parent compounds or do not reach levels high enough to cause observable toxic effects.

### Task 2: Acute Toxicity of the Aqueous and Benzene Fractions of Red Water, Condensate Wastewater, and a Solution of 50 Percent Photolyzed 2,4-DNT

Table 6 presents the acute toxicity estimates computed with the data from the range-finding tests on the aqueous and benzene fractions of red water, condensate wastewater, and a solution of 2,4-DNT that was exposed to filtered UV light until 50 percent of the 2,4-DNT was photolyzed. The concentrations of total dissolved solids (TDS) and 2,4-DNT or 2,4,6-TNT in the fractions are also presented. Because quantities of these materials were limited, the fish bioassays were performed with two fish in 2 L of test solution at each concentration level. Tests with daphnids were not replicated and used five daphnids per beaker.

Table 6. ACUTE TOXICITY OF THE AQUEOUS AND BENZENE FRACTIONS OF TWO TNT WASTEWATERS AND A PARTIALLY PHOTOLYZED SOLUTION OF 2,4-DNT TO D. MAGNA AND FATHEAD MINNOWS

| Test Material | 48-Hour LC50[a] (D. Magna) | | 96-Hour LC50[a] (Minnow) | | TDS mg/L | TNT or DNT[b] mg/L |
|---|---|---|---|---|---|---|
| Red Water | | | | | | |
| Aqueous Fraction | >500 | (20%)[c] | 224 | | 76,197 | <1 |
| Benzene Fraction | 34 | | 22 | | 296 | 4 |
| Condensate | | | | | | |
| Aqueous Fraction | >142 | (40%) | >7.5 | ( 0%) | 1,150 | 1.1 |
| Benzene Fraction | 17 | | >7.5 | (20%) | 275 | 62 |
| Photolyzed 2,4-DNT | | | | | | |
| Aqueous Fraction | >50 | (40%) | 35 | | 50 | 6.7 |
| Benzene Fraction | >50 | ( 0%) | 22 | | 17 | 80 |

[a]Expressed in mg/L TDS.

[b]Red water fractions were analyzed for TNT; the fractions of the other materials were analyzed for DNT.

[c]Percentage of daphnids and minnows killed at the highest tested concentration are shown in parentheses.

In many of the tests, the concentrations used were not high enough to affect at least 50 percent of the organisms. The acute toxicity values derived from such tests are preceded by a "greater than" symbol (>), and the percentage of animals affected is shown in parentheses after the toxicity values.

The tests provided fairly clear evidence that the benzene fractions of red water and condensate wastewater contained components that are more toxic than those in the aqueous fractions; however, they did not reveal much of a difference in the toxicity of the two fractions of 50 percent photolyzed 2,4-DNT.

We concluded that the benzene-extractable (nonpolar) components of red water and condensate wastewater contribute most to the acute toxicity of these wastewaters, and that the water-soluble (polar) and the benzene-extractable (nonpolar) phototransformation products of 2,4-DNT are similar in toxicity.

Task 3: Acute Toxicity of Selected Organic Components of Condensate Wastewater

Table 7 presents, for 32 condensate wastewater components, the 48-hour LC50s and 96-hour LC50s derived from the results of the acute tests with D. magna and the fathead minnow. These toxicity estimates are based on nominal concentrations.

The primary objective of Task 3 was to identify the components with LC50s of 1.0 mg/L or less for inclusion in the synthetic wastewater formulation. Only two compounds showed this property. They were 2,3,6-trinitrotoluene and 2-amino-3,6-dinitrotoluene. Others that were reasonably close (by a factor of 2) were 2,3-, 2,5-, and 3,4-dinitrotoluene, and 1,3,5-trinitrobenzene. From the data of Spanggord and coworkers (1978), it was computed that, on the average, these six compounds account for only 5.2 percent of the total concentration of all 32 compounds in condensate wastewater. This suggested that they may not contribute significantly to the toxicity of the wastewater. To estimate the relative contribution of each compound to the overall toxicity of the wastewater, their toxic unit values were computed.

The toxic unit concept was first described by Sprague and Ramsey (1965) as a method for predicting the toxicity of a mixture of compounds from acute toxicity data on the individual compounds. The toxic unit value of a compound is the ratio of its actual concentration in the mixture and its lethal threshold concentration. According to Sprague (1970), if the sum of the toxic unit values of all components is 1.0 or more, the mixture should be lethal to the organism used in determining the lethal threshold concentrations of the components.

Table 8 presents the toxic unit values computed for the 30 condensate wastewater components. The average concentrations of the compounds in authentic condensate wastewater were used to compute the values. The lethal threshold concentrations were not estimated, so the 48-hour LC50s (Daphnia) and 96-hour LC50s (minnows), which are usually proportional but higher than the lethal threshold concentrations were used. The compounds are ranked in order of the toxic unit values computed from the results of the tests with the fathead minnow, and the discussion below is limited to those values.

Table 7. ACUTE TOXICITY TO FATHEAD MINNOWS AND DAPHNIA MAGNA OF ORGANIC COMPOUNDS IDENTIFIED IN CONDENSATE WASTEWATER

| Compound | Daphnia magna 48-Hour LC50 (mg/L) | | Fathead Minnow 96-Hour LC50 (mg/L) | |
|---|---|---|---|---|
| Toluene | 20.2 | (8.3 - 40.5) | 12.6 | (11.0 - 13.4) |
| 2-Nitrotoluene | >77.1 | (N.A.)[a] | 37.1 | (34.6 - 39.9) |
| 3-Nitrotoluene | 28.1 | (25.3 - 31.6) | 32.5 | (29.9 - 34.7) |
| 4-Nitrotoluene | 11.8 | (9.5 - 19.0)[b] | 49.7 | (46.2 - 52.6)[c] |
| 3-Nitrobenzonitrile | 40.5 | (24.1 - 96.4)[b] | 60.4 | (51.7 - 68.4)[c] |
| 4-Nitrobenzonitrile | 49.3 | (43.7 - 52.8) | 23.8 | (18.9 - 27.7) |
| 2-Amino-4-nitrotoluene | 22.4 | (9.8 - 49.0) | 68.8 | (64.2 - 74.2) |
| 2-Amino-6-nitrotoluene | 14.1 | (11.7 - 16.3) | 50.0 | (45.9 - 53.9)[c] |
| 3-Amino-4-nitrotoluene | 6.6 | (4.9 - 9.7) | 24.2 | (21.8 - 27.4)[c] |
| 4-Amino-2-nitrotoluene | 14.0 | (11.3 - 17.3) | 25.8 | (23.4 - 28.6) |
| 3-Methyl-2-nitrophenol | 19.0 | (13.4 - 22.4) | 46.0 | (43.0 - 49.9) |
| 5-Methyl-2-nitrophenol | 21.5 | (14.4 - 23.4) | 46.9 | (35.7 - 52.5) |
| 1,3-Dinitrobenzene | 49.6 | (42.5 - 59.2) | 7.0 | (5.8 - 8.1) |
| 2,3-Dinitrotoluene | 4.7 | (3.0 - 5.9) | 1.8 | (1.5 - 2.1) |
| 2,4-Dinitrotoluene | 47.5 | (29.5 - 99.7) | 32.8 | (27.3 - 38.0) |
| 2,5-Dinitrotoluene | 3.1 | (2.2 - 3.8) | 1.3 | (1.1 - 1.4) |
| 2,6-Dinitrotoluene | 21.8 | (19.3 - 24.6) | 18.5 | (17.2 - 20.2) |
| 3,4-Dinitrotoluene | 3.7 | (0.96 - 5.4) | 1.5 | (1.1 - 1.8) |
| 3,5-Dinitrotoluene | 45.2 | (42.4 - 48.4) | 22.6 | (13.4 - 27.1) |
| 3,5-Dinitroaniline | 15.4 | (13.5 - 18.0) | 21.8 | (19.1 - 31.3) |
| 1,5-Dimethyl-2,4-DNB | 24.2 | (17.6 - 26.1) | 8.0 | (7.5 - 8.5) |
| 2-Amino-3,6-DNT | 2.5 | (0.65 - 6.5) | 0.9 | (0.7 - 1.2) |
| 2-Amino-4,6-DNT | 4.6 | (3.5 - 5.8) | 15.1 | (14.0 - 16.4) |
| 3-Amino-2,4-DNT | 9.6 | (5.5 - 11.0)[c] | 12.2 | (10.8 - 13.4) |
| 3-Amino-2,6-DNT | 4.8 | (3.5 - 5.7) | 10.9 | (9.5 - 12.3) |
| 4-Amino-2,6-DNT | 5.4 | (3.5 - 9.8) | 6.9 | (5.6 - 8.1) |
| 4-Amino-3,5-DNT | >13.0 | (N.A.) | >13.0 | (N.A.) |
| 5-Amino-2,4-DNT | 3.3 | (3.1 - 3.9) | 2.4 | (1.9 - 30.0) |
| 2,4-Dinitro-5-methylphenol | 3.5 | (3.1 - 3.9)[d] | 3.2 | (2.3 - 4.1)[d] |
| 1,3,5-Trinitrobenzene | 2.7 | (2.4 - 3.1)[d] | 1.1 | (1.0 - 1.2)[d] |
| 2,3,6-Trinitrotoluene | 0.77 | (0.42 - 1.2)[d] | 0.11 | (0.10 - 0.13)[d] |
| 2,4,6-Trinitrotoluene | 11.9 | (11.1 - 12.8)[d] | 3.0 | (2.8 - 3.3)[d] |

[a]N.A., not applicable.

[b]Test solutions aerated.

[c]Control mortality ⩾20 percent.

[d]From Volume I.

Table 8. CONTRIBUTION OF SELECTED INDIVIDUAL ORGANIC COMPONENTS TO THE ACUTE TOXICITY OF CONDENSATE WASTEWATER

| Compound | Average Concentration in Authentic Wastewater (mg/L) | Toxic Units[a] | |
|---|---|---|---|
| | | Fathead Minnow | *D. magna* |
| 2,3,6-Trinitrotoluene | 0.268 | 2.436 | 0.348 |
| 1,3-Dinitrobenzene | 4.000 | 0.571 | 0.081 |
| 2,4-Dinitrotoluene | 14.700 | 0.448 | 0.310 |
| 2,6-Dinitrotoluene | 7.300 | 0.424 | 0.335 |
| 3,4-Dinitrotoluene | 0.500 | 0.333 | 0.135 |
| 5-Amino-2,4-dinitrotoluene | 0.700 | 0.321 | 0.212 |
| 2,5-Dinitrotoluene | 0.400 | 0.308 | 0.085 |
| 2,3-Dinitrotoluene | 0.400 | 0.222 | 0.085 |
| 1,3,5-Trinitrobenzene | 0.153 | 0.139 | 0.057 |
| 2,4,6-Trinitrotoluene | 0.400 | 0.133 | 0.336 |
| 3-Amino-2,4-dinitrotoluene | 1.500 | 0.123 | 0.156 |
| 3-Amino-2,6-dinitrotoluene | 1.200 | 0.110 | 0.250 |
| 4-Amino-2,6-dinitrotoluene | 0.600 | 0.087 | 0.171 |
| 1,5-Dimethyl-2,4-dinitrobenzene | 0.390 | 0.049 | 0.015 |
| 2-Amino-3,6-dinitrotoluene | 0.030 | 0.033 | 0.012 |
| 2,4-Dinitro-5-methylphenol | 0.085 | 0.027 | 0.025 |
| 3,5-Dinitrotoluene | 0.520 | 0.023 | 0.012 |
| Toluene | 0.200 | 0.016 | 0.010 |
| 4-Amino-3,5-dinitrotoluene | 0.200 | 0.015 | 0.015 |
| 3,5-Dinitroaniline | 0.058 | 0.003 | 0.004 |
| 4-Nitrotoluene | 0.100 | 0.002 | 0.008 |
| 3-Amino-4-nitrotoluene | 0.027 | 0.001 | 0.004 |
| 2-Amino-4,6-dinitrotoluene | 0.002 | 0.001 | 0.004 |
| 2-Nitrotoluene | 0.030 | 0.0008 | 0.0004 |
| 5-Methyl-2-nitrophenol | 0.032 | 0.0007 | 0.0015 |
| 2-Amino-4-nitrotoluene | 0.033 | 0.0005 | 0.0015 |
| 4-Nitrobenzonitrile | 0.009 | 0.0004 | 0.0002 |
| 3-Methyl-2-nitrophenol | 0.012 | 0.0003 | 0.0006 |
| 3-Nitrobenzonitrile | 0.013 | 0.0002 | 0.0003 |
| 2-Amino-6-nitrotoluene | 0.010 | 0.0002 | 0.0007 |

[a] Toxic unit = $\frac{\text{Average Concentration in Authentic Wastewater}}{\text{48- or 96-hour LC50}}$

Only one compound—2,3,6-trinitrotoluene—had a toxic unit value of at least 1.0. Its toxic unit value was 2.436, which shows that its concentration in condensate wastewater averages over twice the estimated 96-hour LC50 in fathead minnows. This compound could be the major cause of the toxicity of condensate wastewater, even though it accounts for less than 1 percent of the total concentration of the organic compounds in the wastewater. Eleven other compounds had toxic unit values of at least 0.1. These compounds probably contribute in part to the toxicity of the wastewater because the sum of the toxic unit values (3.13) is greater than 1.0. The remaining 18 compounds had toxic unit values of less than 0.1, and the majority of these had toxic unit values of less than 0.01. It is unlikely that these 18 compounds contribute significantly to the acute toxicity of the wastewater to aquatic organisms, individually or as a group, because the sum of their toxic unit values is considerably less than 1.0.

When the results of the toxicity tests on the 30 compounds were evaluated it was found that 2,3-, 2,5-, and 3,5-DNT were much more toxic than 2,4-, 2,6-, and 3,5-DNT to the daphnids and minnows. The nitro groups of the three more toxic isomers are either ortho or para to each other, whereas the nitro groups in the less toxic isomers are meta to each other.

This relationship between the position of nitro groups on the benzene ring and toxicity appears to hold for other compounds. The 2,4,6-TNT had a 96-hour LC50 in minnows of 3.0 mg/L; its nitro groups are meta oriented. On the other hand, the 96-hour LC50 for 2,3,6-TNT was 0.11 mg/L; its nitro groups are ortho or para oriented. In the tests with D. magna, 2,3,6-TNT was also more toxic than 2,4,6-TNT. Of the seven isomers of amino-dinitro-toluene evaluated, the most toxic isomer was 2-amino-3,6-DNT. Its nitro groups are para oriented; all of the other isomers have meta oriented nitro groups.

Although, 1,4-dinitrobenzene is not a component of condensate wastewater, its acute toxicity to D. magna and the fathead minnow was determined because of the para orientation of its nitro groups; it should exhibit greater toxicity than 1,3-dinitrobenzene (a condensate wastewater component), whose nitro groups are meta oriented. The respective 48-hour LC50 (Daphnia) and 96-hour LC50 (minnows) of 1,4-dinitrobenzene were 0.51 and 1.7 mg/L, whereas they were 49.6 and 7.0 mg/L for 1,3-dinitrobenzene.

The higher toxicity of the isomers with ortho or para oriented nitro groups may be caused by their greater tendency to participate in nucleophilic displacement reactions than isomers with meta oriented nitro groups. It is uncertain, however, how this chemical property relates to biological activity.

## Phase II: Studies on Synthetic Condensate Wastewater (Condensate Water) and Additional Studies on 2,4-DNT

### Task 1: Establishing A Photoirradiation Endpoint for Condensate Water

Some of the tasks in Phase II entailed performing acute toxicity tests on photolyzed condensate water to determine its toxicity relative to that of nonphotolyzed condensate water, the sensitivity of various aquatic species to it, or the effects of different water quality conditions on its toxicity. The volume of photolyzed condensate water needed to perform these tasks required preparing several batches at different times. It was thus necessary to establish an irradiation endpoint to ensure (to a reasonable extent) that all of the batches were toxicologically similar.

Studies with LAP water (see Volume I) showed that with increasing exposure of that material to filtered UV light, its acute toxicity to D. magna declined to a relatively constant level, even though the concentrations of its original components continued to decline. This region, where exposure to filtered UV light no longer affected the toxicity of LAP water, was named the region of toxicological stability. The irradiation endpoint for LAP water was based on the concentrations of TNT and RDX found in the toxicologically stable samples. The same approach was used to establish the irradiation endpoint for condensate water. Although the approach was applied to all four condensate water blends, only the data obtained with Blend 3 and the final blend are presented.

Table 9 presents the 48-hour LC50s obtained with D. magna for samples of Blend 3 irradiated in the flow-through photolytic reactor at five rates ranging from 6 to 43 mL/min. The samples irradiated at 19, 26, and 43 mL/min were similar in toxicity and significantly ($p \leq 0.05$) more toxic than the samples irradiated at 6 and 12 mL/min. The 48-hour LC50s of the samples irradiated at 6 and 12 mL/min were almost identical and not statistically different from each other. These results suggested that toxicologically stable photolyzed condensate water could be prepared by irradiating condensate water at flow rates between 6 and 12 mL/min, provided that all other conditions (e.g., UV light intensity, concentration of the various components in the condensate blend) are constant.

Table 9. ACUTE TOXICITY TO DAPHNIA MAGNA OF BLEND 3 OF CONDENSATE WATER IRRADIATED AT DIFFERENT FLOW RATES

| Flow Rate (mL/min) | 48-Hour LC50 and 95% Confidence Limits (mg/L) | |
|---|---|---|
| 6 | 27.5 | (24.8 - 30.2) |
| 12 | 27.0 | (20.0 - 30.0) |
| 19 | 15.9 | (12.7 - 21.2) |
| 26 | 15.6 | (13.0 - 18.1) |
| 43 | 14.1 | (12.2 - 16.2) |

The final blend of condensate water did not show as pronounced a change in toxicity at the lower flow rates as Blend 3 (Table 10). However, the LC50s followed a trend similar to that obtained with Blend 3.

Table 10. ACUTE TOXICITY TO DAPHNIA MAGNA OF THE FINAL BLEND OF CONDENSATE WATER IRRADIATED AT DIFFERENT FLOW RATES

| Flow Rate (mL/min) | 48-Hour LC50 and 95% Confidence Limits (mg/L) | |
|---|---|---|
| 8 | 19.7 | (14.6 - 28.0) |
| 12 | 18.5 | ( 2.4 - 31.5) |
| 18 | 12.2 | ( 8.7 - 15.0) |
| 30 | 10.8 | ( 7.8 - 13.0)[a] |
| 50 | 13.1 | (10.6 - 15.5) |

[a]Control mortality ⩾20%.

Tables 11 and 12, respectively, present the concentrations of the 30 compounds in Blend 3 and the final blend before irradiation, and of most of the compounds after irradiation at the various flow rates. All of the compounds measured showed some degree of photolysis, and except with 1,3,5-trinitrobenzene, the degree of photolysis increased as the flow rate decreased. This inverse relationship reflects the greater reactor residence time of the sample at the lower flow rates.

Three compounds were resistant to photolysis. These were the two nitrobenzonitriles and 1,3-dinitrobenzene. In the final blend, over 90 percent of the initial concentrations of these compounds remained unphotolyzed. Relatively resistant compounds (less than 50 percent photolysis) were: 3-amino-4-nitrotoluene, 3-amino-2,4-dinitrotoluene, and 5-amino-2,4-dinitrotoluene.

Although 1,3,5-trinitrobenzene exhibited some photolability, the data indicate that it was also synthesized during the irradiation process. In the sample of the final blend irradiated at 50 mL/min, the initial concentration of the compound was reduced by 62 percent, showing that it is photolabile. However, as the irradiation flow rate was reduced from 30 to 12 mL/min, the concentration of 1,3,5-trinitrobenzene increased from 80 percent of its initial concentration to 234 percent more than its initial concentration. In the sample irradiated at 8 mL/min, the concentration of 1,3,5-trinitrobenzene was lower than in the sample irradiated at 12 mL/min; however, it still exceeded its concentration in the non-irradiated sample by 182 percent. Although the concentration of this compound in non-irradiated condensate water was about 50 percent of its 96-hour LC50 in minnows, it increased to the 96-hour LC50 after condensate water was irradiated.

Table 11. CHANGES IN THE RELATIVE CONCENTRATION OF COMPOUNDS IN BLEND 3 OF CONDENSATE WATER AFTER UV IRRADIATION AT DIFFERENT FLOW RATES

| Compound | Concentration (mg/L) Before Irradiation | | Concentration (mg/L) of Blend 3 at Flow Rates (mL/min) of: | | | | |
|---|---|---|---|---|---|---|---|
| | Nominal | Actual | 43 | 26 | 19 | 12 | 6 |
| Toluene | 0.6 | NA[a] | NA | NA | NA | NA | NA |
| 2-Nitrotoluene | 0.09 | 0.09 | 0.037 | 0.029 | 0.021 | 0.007 | 0.002 |
| 4-Nitrotoluene | 0.3 | 0.28 | 0.13 | 0.12 | 0.11 | 0.048 | 0.02 |
| 3-Nitrobenzonitrile | 0.01 | 0.01 | --[b] | -- | -- | -- | -- |
| 4-Nitrobenzonitrile | 0.01 | 0.01 | -- | -- | -- | -- | -- |
| 2-Amino-4-NT | 0.03 | 0.04 | 0.028 | 0.024 | 0.024 | 0.02 | 0.016 |
| 2-Amino-6-NT | 0.1 | 0.12 | 0.079 | 0.079 | 0.071 | 0.053 | 0.036 |
| 3-Amino-4-NT | 0.1 | 0.11 | 0.084 | 0.094 | 0.086 | 0.077 | 0.067 |
| 3-Methyl-2-nitrophenol[c] | 0.03 | | | | | | |
| 5-Methyl-2-nitrophenol | 0.06 | 0.1 | 0.07 | 0.08 | 0.07 | 0.06 | 0.04 |
| 1,3-DNB | 12.01 | 11.8 | 10.6 | 11.1 | 11.0 | 10.4 | 10.0 |
| 2,3-DNT[c] | 1.2 | | | | | | |
| 2,4-DNT | 44.1 | 43.0 | 28.2 | 27.5 | 23.2 | 15.0 | 6.6 |
| 2,5-DNT | 1.2 | 1.2 | 0.31 | 0.20 | 0.11 | 0.04 | 0.04 |
| 2,6-DNT | 21.9 | 22.5 | 2.8 | 1.0 | 0.38 | 0.09 | 0.07 |
| 3,4-DNT | 1.5 | 1.4 | 1.15 | 1.33 | 1.26 | 1.08 | 0.85 |
| 3,5-DNT | 1.5 | 1.6 | 1.36 | 1.44 | 1.38 | 1.22 | 1.15 |
| 3,5-Dinitroaniline | 0.01 | -- | -- | -- | -- | -- | -- |
| 1,5-Dimethyl-2,4-DNB | 1.29 | 1.21 | 0.64 | 0.57 | 0.41 | 0.18 | 0.04 |
| 2-Amino-3,6-DNT | 0.09 | 0.09 | -- | -- | -- | -- | -- |
| 2-Amino-4,6-DNT | 0.06 | 0.06 | -- | -- | -- | -- | -- |
| 3-Amino-2,4-DNT | 4.5 | 4.5 | 3.0 | 3.78 | 3.44 | 3.41 | 2.15 |
| 3-Amino-2,6-DNT | 3.6 | 8.2 | 0.42 | 0.38 | 0.16 | 0.032 | 0.016 |
| 4-Amino-2,6-DNT | 1.8 | 1.5 | 0.5 | 0.7 | 0.45 | 0.34 | 0.09 |
| 4-Amino-3,5-DNT | 0.6 | 0.52 | 0.27 | 0.36 | 0.33 | 0.19 | 0.14 |
| 5-Amino-2,4-DNT | 2.1 | 1.8 | 0.63 | 1.26 | 0.77 | 0.97 | 0.61 |
| 2,4-Dinitro-5-methylphenol | 0.14 | NA | NA | NA | NA | NA | NA |
| 1,3,5-TNB | 0.02 | NA | NA | NA | NA | NA | NA |
| 2,3,6-TNT | 0.06 | NA | NA | NA | NA | NA | NA |
| 2,4,6-TNT | 1.2 | NA | NA | NA | NA | NA | NA |

[a]NA = Not analyzed.

[b]Concentration too low to quantitate.

[c]Peaks were not resolved; both compounds analyzed as one.

Table 12. CHANGES IN THE RELATIVE CONCENTRATIONS OF COMPOUNDS IN THE FINAL BLEND OF CONDENSATE WATER AFTER UV IRRADIATION AT DIFFERENT FLOW RATES

| Compound | Concentration (mg/L) Before Irradiation | | Concentration (mg/L) of the Final Blend at Flow Rates (mL/min) of: | | | | |
|---|---|---|---|---|---|---|---|
| | Nominal | Actual | 50 | 30 | 18 | 12 | 8 |
| Toluene | 0.59 | NA[a] | NA | NA | NA | NA | NA |
| 2-Nitrotoluene | 0.089 | 0.06 | 0.04 | 0.027 | 0.02 | 0.008 | -- |
| 4-Nitrotoluene | 0.295 | 0.22 | 0.17 | 0.13 | 0.10 | 0.008 | 0.043 |
| 3-Nitrobenzonitrile | 0.035 | 0.026 | 0.022 | 0.027 | 0.022 | 0.026 | 0.024 |
| 4-Nitrobenzonitrile | 0.027 | 0.024 | 0.022 | 0.020 | 0.021 | 0.024 | 0.022 |
| 2-Amino-4-NT | 0.091 | 0.09 | 0.031 | 0.05 | 0.047 | 0.031 | 0.023 |
| 2-Amino-6-NT | 0.03 | 0.03 | --[b] | -- | -- | -- | -- |
| 3-Amino-4-NT | 0.08 | 0.08 | 0.08 | 0.075 | 0.085 | 0.074 | 0.058 |
| 3-Methyl-2-nitrophenol[c] | 0.035 | | | | | | |
| 5-Methyl-2-nitrophenol | 0.094 | NA | NA | NA | NA | NA | NA |
| 1,3-DNB | 11.803 | 11.9 | 11.7 | 12.0 | 12.1 | 11.7 | 11.4 |
| 2,3-DNT[c] | 1.18 | | | | | | |
| 2,4-DNT | 43.377 | 47.2 | 43.9 | 42.5 | 35.4 | 29.3 | 17.9 |
| 2,5-DNT | 1.18 | 1.1 | 0.5 | 0.23 | 0.065 | -- | -- |
| 2,6-DNT | 21.541 | 22.4 | 4.9 | 2.08 | 0.34 | 0.1 | -- |
| 3,4-DNT | 1.475 | 1.49 | 1.71 | 1.58 | 1.59 | 1.55 | 1.34 |
| 3,5-DNT | 1.534 | 1.6 | 1.54 | 1.54 | 1.52 | 1.42 | 1.41 |
| 3,5-Dinitroaniline | 0.171 | 0.16 | 0.12 | 0.12 | 0.10 | 0.10 | 0.54 |
| 1,5-Dimethyl-2,4-DNB | 1.151 | 1.0 | 0.87 | 0.5 | 0.31 | 0.25 | 0.073 |
| 2-Amino-3,6-DNT | 0.089 | 0.11 | 0.107 | 0.097 | 0.085 | 0.062 | 0.059 |
| 2-Amino-4,6-DNT | 0.059 | 0.05 | 0.038 | 0.034 | 0.028 | 0.026 | 0.018 |
| 3-Amino-2,4-DNT | 4.426 | 4.3 | 4.4 | 3.8 | 3.6 | 3.3 | 3.0 |
| 3-Amino-2,6-DNT | 3.541 | 3.7 | 1.3 | 0.63 | 0.14 | -- | -- |
| 4-Amino-2,6-DNT | 1.77 | 1.8 | 1.1 | 0.76 | 0.41 | 0.32 | -- |
| 4-Amino-3,5-DNT | 0.59 | 0.54 | 0.61 | 0.43 | 0.36 | 0.28 | 0.27 |
| 5-Amino-2,4-DNT | 2.066 | 2.2 | 2.5 | 2.3 | 1.85 | 1.5 | 1.3 |
| 2,4-Dinitro-5-methylphenol | 0.251 | NA | NA | NA | NA | NA | NA |
| 1,3,5-TNB | 0.451 | 0.5 | 0.19 | 0.40 | 0.64 | 1.17 | 0.91 |
| 2,3,6-TNT | 0.791 | 0.8 | 0.07 | 0.03 | -- | -- | -- |
| 2,4,6-TNT | 1.18 | 1.2 | -- | -- | -- | -- | -- |

[a]NA = Not analyzed.

[b]Concentration too low to quantitate.

[c]Peaks were not resolved; both compounds analyzed as one.

After the toxicity and analytical chemistry data were reviewed it was decided to base the irradiation endpoint for condensate water on the degree of photolysis of 2,4-DNT alone. Thus, whenever a batch of photolyzed condensate water was prepared the concentration of 2,4-DNT only was monitored. Photolyzed condensate water was defined as the final blend with an initial 2,4-DNT concentration of about 44 mg/L and a final concentration of 10mg/L ± 10 percent. The reactor flow rate needed to meet this requirement was 7 to 10 ml/min.

Task 2: Acute Toxicity of Condensate Water and 2,4-DNT to Organisms from Different Trophic Levels

The objective of Task 2 was to determine the range of tolerance of organisms from three trophic levels to 2,4-DNT and condensate water. Acute toxicity tests were performed with four species each of fish, invertebrates, and algae. The tests on condensate water were performed primarily on Blend 1, which was the current blend when Task 2 was scheduled. When the final blend of condensate water was formulated, it was subjected to a limited evaluation with only one species from each trophic level; the blend was tested before and after photoirradiation.

Table 13 presents the 96-hour LC50s obtained from duplicate static tests with the fathead minnow, bluegill, channel catfish, and rainbow trout for 2,4-DNT and Blend 1 of condensate water.

Table 13. ACUTE TOXICITY OF 2,4-DNT AND BLEND 1 OF CONDENSATE WATER TO FOUR SPECIES OF FISH

| Fish Species | Pooled 96-Hour LC50 and 95% Confidence Limits (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | 2,4-DNT | | | Condensate Water | | |
| Fathead minnow | 28.5 | (26.3 - 32.5) | | 22.0 | (20.8 - 23.4) | |
| Bluegill | 13.5 | (12.1 - 15.1) | | 7.1 | ( 6.2 - 8.2) | [a] |
| Channel catfish | 24.8 | (21.0 - 29.3) | [a] | 17.5 | (16.5 - 18.5) | [a] |
| Rainbow trout | 13.6 | (12.2 - 15.2) | | 7.1 | ( 6.2 - 8.2) | [a] |

[a] Test solutions aerated.

The bluegill and rainbow trout were equally sensitive to 2,4-DNT and significantly ($p \leq 0.05$) more sensitive than either the fathead minnow or channel catfish. The channel catfish was somewhat more sensitive to the compound than the fathead minnow; but not significantly so.

Condensate water was significantly more toxic than 2,4-DNT to all of the fish species. The sensitivity ranking of the four species was about the same as observed with 2,4-DNT; however, there was a larger and statistically significant

difference ($p \leq 0.05$) between the LC50s obtained with the fathead minnow and channel catfish; the catfish was the more sensitive species.

Table 14 presents the results of the tests performed on the same materials using four species of invertebrates. The sensitivities of the tested species to 2,4-DNT followed the order: T. dissimilis > D. magna > H. azteca > L. variegatus. Concentrations up to 83.2 mg/L were not lethal to L. variegatus but resulted in 35 percent mortality to H. azteca. With the exception of T. dissimilis, none of the invertebrates were as sensitive to 2,4-DNT as any of the fish species tested. Condensate water was equally toxic to D. magna, H. azteca, and L. variegatus. It was least toxic to T. dissimilis.

Table 14. ACUTE TOXICITY OF 2,4-DNT AND BLEND 1 OF CONDENSATE WATER TO FOUR SPECIES OF INVERTEBRATES

| Invertebrate Species | Pooled 48-Hour LC50 and 95% Confidence Limits (mg/L) | | | |
|---|---|---|---|---|
| | 2,4-DNT | | Condensate Water | |
| D. magna | 38.3 | (33.6 - 43.8) | 23.7 | (18.2 - 35.5) |
| H. azteca | >83.2 | (N.C.)[a] | 22.8 | (20.0 - 28.3) |
| T. dissimilis | 22.5 | (19.8 - 25.5) | 37.9 | (28.7 - 53.0) |
| L. variegatus | >83.2 | (N.C.) | 24.6 | (22.3 - 28.0) |

[a]N.C., not computed.

Table 15 presents the results of our acute toxicity evaluation of photolyzed condensate water. The tests were performed only with the bluegill and D. magna. The results show that exposure of condensate water to filtered UV light reduced its toxicity by a factor of about 3.5. With both test species, the difference

Table 15. ACUTE TOXICITY TO BLUEGILL AND DAPHNIA MAGNA OF THE FINAL BLEND OF CONDENSATE WATER BEFORE AND AFTER PHOTOIRRADIATION

| Test Material | Pooled 96-Hour LC50[a] in Bluegill | | Pooled 48-Hour LC50[a] in D. magna | |
|---|---|---|---|---|
| Condensate Water (Before irradiation) | 2.7 | (2.1 - 3.2) | 11.0 | (6.7 - 14.1) |
| Condensate Water (After irradiation) | 9.2 | (6.5 - 13.0)[b] | 39.8 | (32.5 - 48.8) |

[a]Estimates are expressed in mg/L.

[b]Test solutions aerated.

between the LC50s was statistically significant. These results indicate that condensate water, and presumably condensate wastewater, do not become more acutely toxic on exposure to filtered UV light. The comparison of Table 15 with Tables 13 and 14 also indicates that the toxicity of the final blend to bluegill and Daphnia is greater than that of Blend 1. This is probably because 2,3,6-TNT was added to the final blend and because the amounts of 1,3,5-TNB and 2,4-dinitro-5-methylphenol are relatively higher.

Tables 16 and 17 summarize the results of the 14-day algal assays of 2,4- DNT. At the concentrations tested, the predominant effect on population growth was inhibition, which ranged up to nearly 100 percent in some species.

S. capricornutum showed significant ($p \leq 0.05$) inhibition of growth at all of the tested concentrations (six, ranging from 0.9 to 94.4 mg/L) after four days of exposure. The degree of inhibition ranged from 37.4 to 99.6 percent. All concentrations from 4.7 (the next to the lowest concentration) to 94.4 mg/L caused at least 98 percent inhibition. The degree of inhibition at 0.9 and 4.7 mg/L decreased from 37.4 and 98.1 percent, respectively on Day 4, to 13.5 and 42.5 percent, respectively on Day 14. This indicated recovery. The degree of inhibition on Day 14 was not statistically significant at 0.9 mg/L but it was significant at 4.7 mg/L.

In the tests with M. aeruginosa, we used six concentrations ranging from 0.05 to 10.0 mg/L. All concentrations from 0.5 to 10.0 mg/L caused statistically significant growth inhibition on Days 4 and 14. The degree of inhibition increased with the concentration of 2,4-DNT in the nutrient medium and ranged from 4.2 to 99.5 percent on Day 14. Between Days 4 and 14, recovery occurred at 0.5 and 1.0 mg/L, but at 5.0 and 10.0 mg/L, the degree of inhibition increased.

A. flos-aquae showed significant growth inhibition at all tested concentrations (six, ranging from 0.9 to 94.4 mg/L) except 4.7 mg/L (the second lowest tested). At least 95 percent inhibition occurred at concentrations of 23.3 mg/L or more. With this alga, cell counts were performed only on Day 14.

N. pelliculosa showed about 5 percent growth stimulation at the two lowest concentrations (1.0 and 4.9 mg/L), but this effect was not statistically significant. At all other concentrations (9.8 to 98 mg/L), inhibition was significant and ranged from 91.0 to 97.5 percent. With this alga, cell counts were performed only on Day 14.

Table 18 summarizes the results of the algal assays on Blend 1 of condensate water. With M. aeruginosa, poor growth occurred in one of the three control flasks. The outlier test did not reject the data from that flask. Because of the large variance, our test for significance failed to show significant effects even when the degree of inhibition was as high as 98.1 percent. In all of the tests, six concentrations ranging from 2 to 200 mg/L were used.

On days 4 and 14, S. capricornutum showed statistically significant inhibition at all concentrations except the lowest. At the second lowest concentration (10 mg/L), we observed about 99 percent inhibition on Day 4 and about 87 percent inhibition on Day 14. Almost 100 percent inhibition was observed at 20 to 200 mg/L on both days.

Table 16. EFFECT OF 2,4-DNT ON POPULATION GROWTH OF S. CAPRICORNUTUM AND M. AERUGINOSA EXPOSED UNDER STATIC CONDITIONS FOR 4 AND 14 DAYS

| Algal Species | Concentration of 2,4-DNT mg/L | Percentage of Effect[a] 4 Days | 14 Days |
|---|---|---|---|
| S. capricornutum | 0.9 | -37.4[b] | -13.5 |
| | 4.7 | -98.1[b] | -42.5[b] |
| | 9.4 | -99.5[b] | -99.5[b] |
| | 23.6 | -99.6[b] | -99.8[b] |
| | 47.2 | -99.5[b] | -99.8[b] |
| | 94.4 | -99.6[b] | -99.8[b] |
| M. aeruginosa | 0.05 | 0.0 | - 4.2 |
| | 0.1 | -20.2 | -13.8 |
| | 0.5 | -57.2[b] | -33.4[b] |
| | 1.0 | -65.8[b] | -48.5[b] |
| | 5.0 | -81.3[b] | -97.8[b] |
| | 10.0 | -80.5[b] | -99.5[b] |

[a]Relative to controls: Negative sign denotes inhibition.

[b]Statistically different from controls at the 5 percent level of significance.

Table 17. EFFECT OF 2,4-DNT ON POPULATION GROWTH OF A. FLOS-AQUAE AND N. PELLICULOSA EXPOSED UNDER STATIC CONDITIONS FOR 14 DAYS

| Algal Species | Concentration of 2,4-DNT mg/L | Percentage of Effect at 14 Days[a] |
|---|---|---|
| A. flos-aquae | 0.9 | -23.4[b] |
| | 4.7 | -17.3 |
| | 9.4 | -24.8[b] |
| | 23.6 | -98.7[b] |
| | 47.2 | -99.2[b] |
| | 94.4 | -99.3[b] |
| N. pelliculosa | 1.0 | + 5.1 |
| | 4.9 | + 5.1 |
| | 9.8 | -91.0[b] |
| | 24.5 | -97.8[b] |
| | 49.0 | -97.8[b] |
| | 98.0 | -97.5[b] |

[a]Relative to controls: Positive sign denotes stimulation; negative sign denotes inhibition.

[b]Statistically different from controls at the 5 percent level of significance.

Table 18. EFFECT OF CONDENSATE WATER (BLEND 1) ON POPULATION GROWTH OF FOUR SPECIES OF ALGAE EXPOSED UNDER STATIC CONDITIONS FOR UP TO 14 DAYS

| Algal Species and Exposure Time | Percentage of Effect[a] at Each Tested Concentration[b] | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 10 | 20 | 50 | 100 | 200 |
| S. capricornutum | | | | | | |
| 4 days | -22.6 | -99.1[c] | - 99.9[c] | -99.9[c] | -99.8[c] | -99.9[c] |
| 14 days | - 0.9 | -86.8[c] | - 99.8[c] | -99.8[c] | -99.6[c] | -99.7[c] |
| M. aeruginosa | | | | | | |
| 4 days | -76.9 | +31.0 | +108.0 | -94.3[c] | -95.9[c] | -94.2[c] |
| 14 days | -49.6 | -97.5 | - 95.8 | -98.1 | -99.8[c] | -99.6[c] |
| A. flos-aquae | | | | | | |
| 14 days | +38.7 | -37.8 | - 94.5[c] | -92.7[c] | -90.7[c] | -96.8[c] |
| N. pelliculosa | | | | | | |
| 14 days | -36.4 | -71.1[c] | - 90.9[c] | -90.6[c] | -88.3[c] | -93.1[c] |

[a]Relative to controls: Positive sign denotes stimulation; negative sign denotes inhibition.

[b]Total nominal concentration of all 17 compounds, expressed in mg/L.

[c]Statistically different from controls at the 5 percent significance level.

The growth of M. aeruginosa was generally inhibited by condensate water. On Day 4, the responses at 10, and 20 mg/L were incongruous: 2, 50, 100, and 200 mg/L inhibited growth, whereas 10 and 20 mg/L stimulated growth by as much as 108 percent. The four-day effect (inhibition) of condensate water on the growth of this alga was statistically significant only at the three highest concentrations. On Day 14, inhibition was observed at all six concentrations. The degree of inhibition ranged from 49.6 to 99.8 percent and increased with the concentration of 2,4-DNT. The 108 percent stimulation observed at 20 mg/L on Day 4 was reversed to 95.8 percent inhibition on Day 14. Probably because of the inconsistent control response mentioned earlier, the inhibitory effect of condensate water was statistically significant only at 100 and 200 mg/L.

The response to condensate water of A. flos-aquae and N. pelliculosa was determined only on Day 14. The compound inhibited the growth of A. flos-aquae at all tested concentrations except at 2 mg/L, where about 39 percent stimulation (not significant) occurred. Cultures exposed to 20 to 200 mg/L of condensate water showed statistically significant inhibition, which ranged from about 91 to about 97 percent. Condensate water inhibited N. pelliculosa at all tested concentrations; inhibition ranged from 36.4 percent at 2 mg/L to 93.1 percent.

Table 19 summarizes the results of the algal assays performed on photolyzed and nonphotolyzed samples of the final condensate water blend using S. capricornutum. No other species of algae was used to evaluate the final blend.

Table 19. EFFECT OF PHOTOLYZED AND NONPHOTOLYZED CONDENSATE WATER (FINAL BLEND) ON THE GROWTH OF S. CAPRICORNUTUM

| Nominal Concentrations Tested (mg/L)[b] | Percentage of Effect[a] | | | |
|---|---|---|---|---|
| | Nonphotolyzed Condensate | | Photolyzed Condensate | |
| | Day 4 | Day 14 | Day 4 | Day 14 |
| 1.0 | -27.4 | - 1.8 | -13.2 | - 4.6 |
| 4.8 | -98.1[c] | -47.3[c] | -92.2[c] | -59.3[c] |
| 9.7 | -99.5[c] | -99.5[c] | -96.7[c] | -66.8[c] |
| 24.2 | -99.6[c] | -99.7[c] | -99.1[c] | -98.7[c] |
| 48.5 | -99.6[c] | -99.5[c] | -99.0[c] | -99.0[c] |
| 97.0 | -99.6[c] | -99.6[c] | -99.1[c] | -99.0[c] |

[a]Relative to controls, negative sign denotes inhibition.

[b]Nominal, sum of the concentrations of all 30 compounds in condensate water before irradiation.

[c]Statistically different from controls at the 5 percent significance level.

Photoirradiation had no appreciable effect on the toxicity of the final blend. Both the photolyzed and nonphotolyzed samples caused statistically significant inhibition of growth at all but the lowest concentration (1.0 mg/L).

The environmental implications of algal assay data are difficult to assess. This is particularly true for data derived from static assays in which the nutrient medium and/or the toxicant could become depleted because depletion of the nutrient medium could inhibit growth. Conversely, in the presence of ample nutrients, depletion of the toxicant could cause apparent recovery from any initial inhibition.

The concentration of nutrients in the medium used should have been ample to support growth for 21 days, so the observed effects are assumed to have been caused by the test materials. However, because 2,4-DNT and other components of condensate water are photolabile, the observed effects cannot be related to the tested concentrations with certainty. The data suggest that continued exposure to 10 mg/L of condensate wastewater or 2,4-DNT alone will cause at least 90 percent inhibition of the algal species tested. Recovery could occur if the concentration of either of these materials were to decline towards zero; however, recovery is unlikely at concentrations above 20 mg/L.

### Task 3: Effects of Water Quality on the Acute Toxicity of 2,4-DNT and Condensate Water to the Bluegill

Table 20 presents the 96-hour LC50s obtained with the bluegill for 2,4-DNT tested under three levels of water temperature, hardness, and pH. The bluegill was used in this task because the results from Task 1 of Phase II indicated that it met the species-selection criterion of the general testing plan by being the most sensitive warm water fish species.

Table 20. EFFECTS OF WATER TEMPERATURE, HARDNESS, AND pH ON THE ACUTE TOXICITY OF 2,4-DNT TO THE BLUEGILL

| Water Quality Parameter | Level | | 96-Hour LC50 and 95% Confidence Limits (mg/L) | |
|---|---|---|---|---|
| | Desired | Actual | | |
| Temperature (°C)[a] | 15 | 17.3 | 24.0 | (18.0 - 32.0)[b] |
| | 20 | 20.8 | 12.8 | (11.5 - 14.0)[b] |
| | 25 | 24.5 | 7.8 | ( 7.2 - 8.5)[b] |
| Hardness[c] (mg/L as $CaCO_3$) | 40 | 47.0 | 12.8 | (11.5 - 14.0)[b] |
| | 100 | 109.0 | 18.8 | (17.1 - 20.6)[b] |
| | 250 | 251.0 | 16.4 | (14.8 - 17.9)[b] |
| pH[d] | 6 | 6.3 | 8.4 | ( 7.0 - 10.0)[b] |
| | 7 | 7.1 | 12.8 | (11.5 - 14.0)[b] |
| | 8 | 8.0 | 9.4 | ( 8.7 - 10.4)[b] |

[a]Nominal pH and hardness were 7.0 ($\bar{x}$ = 7.1, range = 7.0-7.2) and 40 mg/L ($\bar{x}$ = 45.7, range = 43.2-47.0), respectively.

[b]Test solutions aerated.

[c]Nominal temperature and pH were 20°C ($\bar{x}$ = 20.7, range = 20.6-20.8) and 7.0 ($\bar{x}$ = 7.2, range = 7.1-7.4), respectively.

[d]Nominal temperature and hardness were 20°C ($\bar{x}$ = 20.3, range = 20.0-20.8) and 40 mg/L ($\bar{x}$ = 41.7, range = 39.0-47.0), respectively.

The toxicity of 2,4-DNT increased significantly with temperature. At 20 and 25°C, the compound was two and three times, respectively, more toxic than at 15°C. The compound was significantly less toxic to bluegills in moderately hard water (109 mg/L as $CaCO_3$) with a pH of about 7 than in soft or very hard water with the same pH. It was also significantly less toxic in soft water when the pH of the water was 7 than when it was 6 or 8.

Table 21 presents the 96-hour LC50s obtained for Blend 1 of condensate water under conditions similar to those just described. Although there were statistically significant differences between some of the LC50s within a group, the differences were not appreciable.

Table 21. EFFECTS OF WATER TEMPERATURE, HARDNESS, AND pH ON THE ACUTE TOXICITY OF CONDENSATE WATER (BLEND 1) TO THE BLUEGILL

| Water Quality Parameter | Level | | 96-Hour LC50 and 95% Confidence Limits (mg/L) | |
|---|---|---|---|---|
| | Desired | Actual | | |
| Temperature (°C)[a] | 15 | 16.0 | 7.0 | (6.3 - 7.8) |
| | 20 | 20.0 | 6.0 | (5.6 - 6.5) |
| | 25 | 24.5 | 6.3 | (5.8 - 6.9) |
| Hardness[b] (mg/L as $CaCO_3$) | 40 | 32 | 6.0 | (5.6 - 6.5) |
| | 100 | 95 | 5.2 | (4.7 - 5.6)[c] |
| | 250[b] | 234 | 5.5 | (5.2 - 5.9)[c] |
| pH[d] | 6 | 6.2 | 5.4 | (5.1 - 5.9) |
| | 7 | 7.0 | 6.0 | (5.6 - 6.5) |
| | 8 | 7.9 | 7.4 | (6.7 - 8.1) |

[a]Nominal pH and hardness were 7.0 ($\bar{x}$ = 7.1, range = 7.0-7.2) and 40 mg/L ($\bar{x}$ = 29.7, range = 25.0-32.0), respectively.

[b]Nominal temperature and pH were 20°C ($\bar{x}$ = 20.1, range = 20.0-20.2) and 7.0 ($\bar{x}$ = 7.2, range = 7.0-7.3), respectively.

[c]Test solutions aerated.

[d]Nominal temperature and hardness were 20°C ($\bar{x}$ = 20.0, range = 20.0-20.0) and 40 mg/L ($\bar{x}$ = 30.7, range = 30.0-32.0), respectively.

Tables 22 and 23, respectively, present for the non-photolyzed and photolyzed final blend of condensate water the 96-hour LC50s obtained under various conditions of water temperature, hardness, and pH.

When the data on Tables 22 and 23 were compared, it was noted that under the same water quality conditions, photolyzed condensate water exhibited less toxicity than non-photolyzed condensate water. On the average, photoirradiation reduced the toxicity of condensate water by a factor of about 2 (1.92). This reduction was much less than observed in Task 2 (see Table 15) where a toxicity reduction factor of 4 was obtained.

Table 22. EFFECTS OF WATER TEMPERATURE, HARDNESS, AND pH ON THE ACUTE TOXICITY OF CONDENSATE WATER (FINAL BLEND) TO THE BLUEGILL

| Water Quality Parameter | Level Desired | Level Actual | 96-Hour LC50 and 95% Confidence Limits (mg/L) |
|---|---|---|---|
| Temperature (°C)[a] | 15 | 16.1 | 7.0 (6.4 - 7.6)[b] |
| | 20 | 20.3 | 5.7 (5.2 - 6.2)[b] |
| | 25 | 25.0 | 5.0 (4.5 - 5.5)[b] |
| Hardness[c] (mg/L as $CaCO_3$) | 40 | 61 | 7.0 (6.4 - 7.6)[b] |
| | 100 | 94 | 7.7 (7.1 - 8.4)[b] |
| | 250 | 244 | 6.2 (5.7 - 6.8)[b] |
| pH[d] | 6 | 6.2 | 2.6 (2.1 - 3.2)[b] |
| | 7 | 7.1 | 5.7 (5.2 - 6.2)[b] |
| | 8 | 7.9 | 5.4 (5.0 - 5.9)[b] |

[a]Nominal pH and hardness were 7.0 ($\bar{x}$ = 7.3, range = 7.1-7.4) and 40 mg/L ($\bar{x}$ = 79, range = 61-88), respectively.

[b]Test solutions aerated.

[c]Nominal temperature and pH were 20°C ($\bar{x}$ = 20.7, range = 20.2-20.9) and 7.0 ($\bar{x}$ = 7.3, range = 7.1-7.6), respectively.

[d]Nominal temperature and hardness were 20°C ($\bar{x}$ = 20.4, range = 20.3-20.4) and 40 mg/L ($\bar{x}$ = 61, range = 61-61), respectively.

Table 23. EFFECTS OF WATER TEMPERATURE, HARDNESS, AND pH ON THE ACUTE TOXICITY OF PHOTOLYZED CONDENSATE WATER (FINAL BLEND) TO THE BLUEGILL

| Water Quality Parameter | Level Desired | Level Actual | 96-Hour LC50 and 95% Confidence Limits (mg/L) |
|---|---|---|---|
| Temperature (°C)[a] | 15 | 16.4 | 14.1 (10.0 - 20.0)[b] |
| | 20 | 20.0 | 11.3 (9.6 - 13.2)[b] |
| | 25 | 25.1 | 7.1 (5.0 - 10.0)[b] |
| Hardness[c] (mg/L as $CaCO_3$) | 40 | 49 | 11.3 (9.6 - 13.2)[b] |
| | 100 | 110 | 8.8 (7.5 - 10.4) |
| | 250 | 232 | 9.4 (7.9 - 11.0) |
| pH[d] | 6 | 6.0 | 8.5 (7.2 - 10.0)[b] |
| | 7 | 7.0 | 11.3 (9.6 - 13.2)[b] |
| | 8 | 8.0 | 12.8 (11.0 - 14.9)[b] |

[a]Nominal pH and hardness were 7.0 ($\bar{x}$ = 6.9, range = 6.8-6.9) and 40 mg/L ($\bar{x}$ = 34.3, range = 27.0-49.0), respectively.

[b]Test solutions aerated.

[c]Nominal temperature and pH were 20°C ($\bar{x}$ = 20.3, range = 20.0-20.8) and 7.0 ($\bar{x}$ = 7.0, range = 6.9-7.2), respectively.

[d]Nominal temperature and hardness were 20°C ($\bar{x}$ = 19.9, range = 19.8-20.0) and 40 mg/L ($\bar{x}$ = 49.0, range = 49.0-49.0), respectively.

Temperature affected the toxicity of photolyzed condensate water in the same way it affected the toxicity of 2,4-DNT: toxicity increased with temperature. The 96-hour LC50s obtained at 15 and 20°C were not significantly different from each other, but the 96-hour LC50 obtained at 25°C was significantly lower than those obtained at the other two temperatures.

Photolyzed condensate water was more toxic in moderately hard water (110 mg/L as $CaCO_3$) than in soft or very hard water, and also more toxic in water with a pH of 6 than in waters with pHs of 7 or 8.

Although the three water quality parameters studied had statistically significant effects on the toxicity of 2,4-DNT and photolyzed and nonphotolyzed condensate water, the magnitudes of the effects were not appreciable in our opinion. The largest effect (caused by temperature and 2,4-DNT) caused the LC50s to change only by a factor of 3.

Task 4: Acute Toxicity of Condensate Water to Selected Early Life Stages of the Fathead Minnow

Task 4 was performed after Blend 2 of condensate water had been developed, but before Blend 3 was developed. Table 24 shows the 96-hour LC50s obtained with fathead minnow embryos and fry of different ages for photolyzed and nonphotolyzed samples of Blend 2. Photolyzed condensate was prepared by irradiating a sample of Blend 2 containing an initial 2,4-DNT concentration of 30 mg/L until 49.9 mg/L of 2,4-DNT remained.

Table 24. ACUTE TOXICITY OF CONDENSATE WATER AND PHOTOLYZED CONDENSATE WATER (BLEND 2) TO SELECTED EARLY LIFE STAGES OF THE FATHEAD MINNOW

| Life Stage | 96-Hour LC50 and 95% Confidence Limits (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Condensate Water | | | Photolyzed Condensate Water | | |
| Embryo | 20.2 | (16.3 - | 24.8) | 17.8 | (11.7 - | 65.0) |
| 2-Day-old Fry | 3.7 | (2.7 - | 4.9) | 8.2 | (7.3 - | 9.2) |
| 7-Day-old Fry | 7.6 | (5.0 - | 10.0) | 8.5 | (7.2 - | 9.9) |
| 30-Day-old Fry | 8.6 | (7.9 - | 9.3) | 21.5 | (19.0 - | 23.9) |
| 60-Day-old Fry | 6.5 | (2.6 - | 9.9) | 17.4 | (14.9 - | 20.0) |

Condensate water was least toxic to the embryo and most toxic to the two-day-old fry. The 96-hour LC50s obtained with the embryo and the 2-day-old fry were significantly higher and lower, respectively, than those obtained with the 7-, 30-, and 60-day-old fry, but the differences between the 96-hour LC50s obtained with these older fry were not significant. The high tolerance of the embryo was also observed in similar tests on photolyzed and nonphotolyzed LAP water (see Volume I).

Although the embryo was among the most tolerant to photolyzed condensate water of the five stages, it was not as outstandingly tolerant as it was with the other materials tested. We believe that there may have been something wrong with the embryos used in the test because control mortality amounted to 10 percent and solvent (acetone) control mortality amounted to 30 percent. The two and seven-day-old fry were the least tolerant of the stages tested; the 96-hour LC50s obtained with these two stages were not significantly different.

The LC50s for photolyzed condensate water were generally higher than for nonphotolyzed condensate water with similar life stages. The exception was the LC50 obtained for photolyzed condensate wastewater with the embryo. This supports our belief that the embryos used to evaluate photolyzed condensate water were in a poor state of health.

Task 5: Exploratory Bioconcentration Studies on 2,4-DNT

In the exploratory bioconcentration tests, four groups of aquatic species were exposed for four days to radiolabelled 2,4-DNT alone and as a component of condensate water (Blend 3). A detailed description of our test procedures is presented in Volume I (Liu et al., 1980). The alga and the invertebrates were subjected to an exposure phase only and were sampled and radioanalyzed on Day 4. The bluegills were subjected to a 10-day depuration phase, and individuals were removed and radioanalyzed on Days 2 and 4 of the exposure phase and on Days 3 and 10 of the depuration phase. Table 25 presents the results of the tests.

The four-day bioconcentration factors (BCF), which were computed by dividing the radioactivity/gram of organism by the radioactivity/gram of water, were less than 100 for all three animal species. The depuration data obtained with the bluegill tissues indicates that 2,4-DNT or its metabolites are rapidly excreted. Nearly 100 percent of the radioactivity found in the tissues of the bluegill on Day 4 had disappeared from the tissues three days after the fish were placed in clean water. The alga sorbed a considerable amount of labelled 2,4-DNT. The 4-day BCFs obtained with the alga were 2507 for 2,4-DNT alone and 2149 for 2,4-DNT in condensate water.

There are no recognized criteria for determining when a BCF portends problems. The presence of a compound in the tissues of an organism does not necessarily indicate that the compound is hazardous. Generally, BCF values of 10 or less and often even up to 100 are considered no cause for alarm. However, BCFs greater than 100, and especially those greater than 1000, suggest possible problems for human consumption and the need for further study (ASTM, 1978). These unofficial guidelines refer to the steady-state BCF, which is the maximum attainable in a species exposed to a particular concentration of the test material, and to BCFs obtained with fish.

Our study showed that during the four-day exposure period, only the algae concentrated 2,4-DNT extensively. We do not know, however, whether the compound was absorbed or adsorbed to the algal cells. The compound was not sorbed extensively by the animal species, and the amount sorbed by bluegills was excreted. These observations suggest that the bioconcentrating potential of 2,4-DNT in aquatic animals is low.

Table 25. UPTAKE OF 2,4-DNT IN CONDENSATE WATER AND 2,4-DNT ALONE BY SELECTED AQUATIC ORGANISMS, PHASE 1, TASK 5

| Compound | Organism | Water[a] (dpm/g) | Tissue (dpm/g) | | BCF[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Uptake | | Depuration | |
| | | | 2 days | 4 days | 2 days | 4 days | 3 days | 10 days |
| 2,4-DNT | Selenastrum | 282 | — | 707,058 | — | 2,507 | — | — |
| | Daphnia | 376 | — | 5,074 | — | 13 | — | — |
| | Lumbriculus | 354 | — | 20,696 | — | 58 | — | — |
| | Bluegill | | | | | | | |
| | Viscera | 341 | 18,159 | 26,553 | 53 | 78 | 1 | 0 |
| | Muscle | 341 | 1,423 | 1,542 | 4 | 4 | 0 | 0 |
| Condensate | Selenastrum | 280 | — | 601,823 | — | 2,149 | — | — |
| | Daphnia | 316 | — | 4,582 | — | 14 | — | — |
| | Lumbriculus | 321 | — | 20,170 | — | 63 | — | — |
| | Bluegill | | | | | | | |
| | Viscera | 318 | 15,081 | 26,602 | 47 | 84 | 0 | 2 |
| | Muscle | 318 | 1,566 | 1,542 | 5 | 5 | 0 | 0 |

[a] Initial disintegration per gram (dpm/g).

[b] Bioconcentration factor: $\frac{\text{dpm per gram of tissue}}{\text{dpm per gram of water.}}$

Table 26. STEADY-STATE BCF VALUES CALCULATED FROM ESTIMATED LOG P VALUES FOR 30 CONDENSATE WASTEWATER COMPOUNDS

| Compound | Estimated Log P | BCF |
|---|---|---|
| 3,5-Dinitrotoluene | 0.395 | 1.18 |
| 3-Amino-2,4-dinitrotoluene | 1.06 | 3.76 |
| 3-Amino-2,6-dinitrotoluene | 1.06 | 3.76 |
| 5-Amino-2,4-dinitrotoluene | 1.06 | 3.76 |
| 4-Amino-2,6-dinitrotoluene | 1.06 | 3.76 |
| 4-Amino-2,5-dinitrotoluene | 1.06 | 3.76 |
| 2-Amino-3,6-dinitrotoluene | 1.06 | 3.76 |
| 2-Amino-4,6-dinitrotoluene | 1.06 | 3.76 |
| 3-Nitrobenzonitrile | 1.17 | 4.56 |
| 4-Nitrobenzonitrile | 1.19 | 4.72 |
| 2-Amino-6-nitrotoluene | 1.32 | 5.93 |
| 3-Amino-4-nitrotoluene | 1.32 | 5.93 |
| 2-Amino-4-nitrotoluene | 1.32 | 5.93 |
| 1,3,5-Trinitrobenzene | 1.36 | 6.36 |
| 2,4-Dinitro-5-methylphenol | 1.62 | 10.03 |
| 1,3-Dinitrobenzene | 1.62 | 10.03 |
| 5-Methyl-2-nitrophenol | 1.88 | 15.80 |
| 3-Methyl-2-nitrophenol | 1.88 | 15.80 |
| 2,4,6-Trinitrotoluene | 2.03 | 20.54 |
| 2,3,6-Trinitrotoluene | 2.03 | 20.54 |
| 2,4-Dinitrotoluene | 2.28 | 31.83 |
| 2,6-Dinitrotoluene | 2.28 | 31.83 |
| 3,5-Dinitrotoluene | 2.28 | 31.83 |
| 3,4-Dinitrotoluene | 2.28 | 31.83 |
| 2,3-Dinitrotoluene | 2.28 | 31.83 |
| 2,5-Dinitrotoluene | 2.28 | 31.83 |
| 2-Nitrotoluene | 2.30 | 32.96 |
| 4-Nitrotoluene | 2.40 | 39.26 |
| Toluene | 2.58 | 53.80 |
| 1,5-Dimethyl-2,4-dinitrobenzene | 2.95 | 102.80 |

To estimate the steady-state BCF for 2,4-DNT and the other components of condensate water, we used the equation of Veith and coworkers (1978), which computes steady-state BCFs from Log P (octanol/water partition coefficient) values. The equation is:

$$\text{Log BCF} = 0.76 \text{ Log P} - 0.23.$$

We calculated the Log P values using the methods of Leo and coworkers (1971). Table 26 presents the Log Ps and the steady-state BCFs computed for the components of condensate water.

The computed BCFs are associated with some degree of uncertainty. The equation supposedly applies to organisms with a lipid content of 8 percent of body weight. The lipid content of the organisms used in the tests was not determined. The computed steady-state BCF for 2,4-DNT was lower than the observed four-day BCFs associated with bluegill viscera, but much higher than that associated with the muscle tissue. Without information on the BCF based on whole fish analysis it is impossible to determine the accuracy of the computed BCF. The computed steady-state BCF for 2,4-DNT does, however, support our conclusions based on the data from the exploratory bioconcentration tests that 2,4-DNT has little propensity for bioaccumulation. With the possible exception of 1,5-dimethyl-2,4-dinitrobenzene, we also believe that none of the other organic components of condensate wastewater are likely to bioconcentrate appreciably.

## Phase III: Definitive Acute Toxicity Studies

### Task 1: Determination of Incipient LC50s for 2,4-DNT and Condensate Water

As explained earlier, Task 1 was the only task of five originally comprising Phase III performed. In this task, duplicated flow-through acute toxicity tests were conducted on 2,4-DNT and the final blend of condensate water using four species of fish and two species of invertebrates.

Table 27 presents the means and standard deviations of the lengths (total) and weights of the fish used in the tests. The weights and lengths were determined on the control fish after each test was completed. All animals were fed once a day during the tests.

Table 27. MEANS AND STANDARD DEVIATIONS OF THE LENGTH AND WEIGHT OF THE CONTROL FISH[a] FROM THE FLOW-THROUGH ACUTE TESTS ON 2,4-DNT AND CONDENSATE WATER

| Test Species | 2,4-DNT | | | | Condensate Water | | | |
|---|---|---|---|---|---|---|---|---|
| | Length (cm) | | Weight (g) | | Length (cm) | | Weight (g) | |
| Fathead minnow | 3.2 | (0.49)[b] | 0.34 | (0.10) | 2.8 | (0.44) | 0.3 | (0.11) |
| Bluegill | 4.8 | (0.45) | 1.21 | (0.30) | 5.3 | (0.56) | 2.0 | (0.55) |
| Channel catfish | 6.1 | (0.32) | 1.98 | (0.39) | 7.7 | (0.88) | 4.2 | (1.31) |
| Rainbow trout | 6.3 | (0.90) | 2.77 | (1.07) | 5.4 | (0.57) | 1.8 | (0.65) |

[a] Weights and measurements from 20 fish per test per species.

[b] Numbers in parentheses represent standard deviations.

Table 28 shows the means and standard deviations of the measured concentrations of 2,4-DNT for all of the tests, and Table 29 shows the same kinds of statistics for the tests performed on condensate water. Tables 30 and 31 summarize the temperature and water quality data from all tests on 2,4-DNT and condensate water, respectively.

The LC50s (24-, 48-, and 96-hour and incipient) obtained for 2,4-DNT with the six test species are presented in Table 32. The numbers in parentheses after the incipient LC50s denote exposure time in hours.

The 48-hour invertebrate LC50s and 96-hour fish LC50s obtained under static conditions (see Tables 13 and 14) were compared with the same LC50s obtained under flow-through conditions; the static LC50s were lower than the flow-through LC50s for all six species. The difference between the two LC50s was statistically significant at the 5 percent level for the minnow, catfish, and D. magna, but not statistically significant for the bluegill and trout. The LC50s obtained with L. variegatus could not be statistically compared.

Whenever the static LC50 of an organic compound is less than its flow-through LC50, its transformation products usually become suspect because the static exposure technique allows them to accumulate in the exposure vessels. Because 2,4-DNT is photolabile, the UV light produced by the fluorescent lamps in the testing room could have caused some photolysis. However, it is unlikely that the greater toxicity of the static solutions was caused by phototransformation products of 2,4-DNT because tests performed in earlier phases of this study showed quite conclusively that the toxicity of the compound is reduced by photoirradiation. It is unlikely that the greater toxicity of the static solutions was caused by biotransformation products. Environmental fate studies performed by SRI under Contract DAMD17-78-C-8081 show that 2,4-DNT is rapidly mineralized by microorganisms. Organic intermediates that have been identified so far are 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene. Neither shows extraordinary acute toxicity to D. magna or fathead minnows (see Table 7) or to microorganisms (Spanggord, et al., 1980), and both are rapidly biotransformed.

That the static solutions were similar or greater in toxicity than the flow-through solutions to all six test species indicates that it is unlikely that the observations were chance events. Although the differences are interesting, we consider them environmentally insignificant. The difference between species sensitivities was considerably greater than the difference in the toxicities of the static and flow-through solutions.

With all six species, the flow-through incipient LC50s were 32 to 77 percent lower ($p \leq 0.05$) than the flow-through 96-hour LC50s. The differences between these LC50s were statistically significant in all cases. Based on the incipient LC50s, the order of sensitivity of the six species is D. magna = rainbow trout > bluegill > channel catfish > fathead minnows = L. variegatus.

Table 33 presents the 24-, 48-, and 96-hour LC50s and the incipient LC50s for the final blend of condensate water. The static and flow-through LC50s were *not compared* because they were determined with different blends. Based on the incipient LC50s, the order of sensitivity of the six tested species is: rainbow trout = bluegill sunfish > fathead minnow > channel catfish = D. magna > L. variegatus.

Table 28. MEANS AND STANDARD DEVIATION (S.D.) OF THE MEASURED CONCENTRATIONS OF 2,4-DNT EVALUATED IN THE FLOW-THROUGH ACUTE TESTS

| Test Species | Nominal Concentration | Measured Concentration (mg/L) Series A N[a] | Series A Mean | Series A S.D. | Series B N[a] | Series B Mean | Series B S.D. |
|---|---|---|---|---|---|---|---|
| Fathead minnow | 10 | 5 | 8.6 | 0.82 | 4 | 8.5 | 0.21 |
| | 20 | 5 | 17.9 | 1.47 | 4 | 18.7 | 1.71 |
| | 30 | 5 | 29.3 | 0.98 | 4 | 28.8 | 2.07 |
| | 40 | 5 | 39.7 | 1.85 | 4 | 40.7 | 1.68 |
| | 50 | 1 | 46.5 | -- | 0 | -- | -- |
| Bluegill | 5 | 3 | 4.3 | 0.12 | 3 | 4.2 | 0.10 |
| | 10 | 6 | 7.8 | 2.53 | 4 | 9.0 | 0.19 |
| | 20 | 5 | 17.9 | 1.89 | 5 | 18.8 | 1.05 |
| | 30 | 3 | 28.9 | 0.38 | 2 | 27.7 | 0.42 |
| | 40 | 1 | 38.2 | -- | 1 | 37.5 | -- |
| Channel catfish | 10 | 4 | 8.1 | 0.67 | 3 | 6.7 | 3.62 |
| | 18 | 4 | 16.0 | 1.21 | 3 | 12.8 | 6.26 |
| | 32 | 4 | 29.6 | 1.33 | 3 | 28.9 | 2.29 |
| | 56 | 1 | 46.0 | -- | 0 | -- | -- |
| | 75 | 1 | 65.0 | -- | 0 | -- | -- |
| Rainbow trout | 1 | 4 | 0.88 | 0.05 | 4 | 0.85 | 0.04 |
| | 5 | 6 | 4.6 | 0.33 | 6 | 4.3 | 0.23 |
| | 10 | 5 | 9.3 | 0.58 | 4 | 10.2 | 0.59 |
| | 15 | 3 | 12.2 | 2.62 | 3 | 15.0 | 1.49 |
| | 20 | 3 | 19.4 | 1.18 | 2 | 18.5 | 0.92 |
| *L. variegatus*[b] | 10 | 8 | 9.6 | 0.53 | | | |
| | 25 | 8 | 23.7 | 1.39 | | | |
| | 50 | 8 | 48.0 | 1.58 | | | |
| | 80 | 2 | 77.6 | 1.56 | | | |
| | 120 | 2 | 127.5 | 3.96 | | | |
| *D. magna*[b] | 1.0 | 7 | 0.73 | 0.042 | | | |
| | 5.6 | 7 | 4.7 | 0.65 | | | |
| | 10.0 | 7 | 7.8 | 0.36 | | | |
| | 24.0 | 4 | 22.2 | 0.50 | | | |
| | 56.0 | 1 | 43.7 | -- | | | |

[a]Number of samples analyzed.

[b]Replicate treatment group A and B were exposed in separate "egg cups" suspended in the same exposure tank.

Table 29. MEANS AND STANDARD DEVIATIONS (S.D.) OF THE MEASURED CONCENTRATIONS[a] OF CONDENSATE WATER EVALUATED IN THE FLOW-THROUGH ACUTE TESTS

| Test Species | Nominal Concentration | Measured Concentration (mg/L) Series A N[b] | Series A Mean | Series A S.D. | Series B N[b] | Series B Mean | Series B S.D. |
|---|---|---|---|---|---|---|---|
| Fathead minnow | 1 | 8 | 0.9 | 0.22 | 8 | 0.6 | 0.26 |
| | 5 | 8 | 4.6 | 0.75 | 8 | 3.7 | 0.58 |
| | 10 | 4 | 9.6 | 1.00 | 5 | 9.5 | 0.97 |
| | 15 | 2 | 15.6 | 1.63 | 3 | 14.9 | 1.65 |
| Bluegill | 1 | 5 | 0.9 | 0.14 | 5 | 0.6 | 0.06 |
| | 5 | 6 | 3.6 | 0.87 | 6 | 3.8 | 0.49 |
| | 10 | 3 | 8.3 | 2.69 | 3 | 7.9 | 2.91 |
| | 15 | 2 | 13.7 | 1.52 | 2 | 14.4 | 0.28 |
| Channel catfish | 1 | 4 | 1.1 | 0.05 | 4 | 1.0 | 0.08 |
| | 5 | 4 | 4.6 | 0.29 | 4 | 4.8 | 0.45 |
| | 10 | 5 | 10.0 | 0.46 | 3 | 10.9 | 0.25 |
| | 15 | 2 | 15.6 | 0.28 | 2 | 15.1 | 0.14 |
| Rainbow trout | 0.5 | 4 | 0.75 | 0.10 | 4 | 0.80 | 0.08 |
| | 1 | 5 | 1.0 | 0.08 | 5 | 1.1 | 0.13 |
| | 5 | 5 | 5.8 | 0.44 | 6 | 5.3 | 0.25 |
| | 8 | 4 | 7.3 | 2.0 | 4 | 7.8 | 1.07 |
| | 10 | 2 | 18.7 | 3.7 | 2 | 15.9 | 1.98 |
| *L. variegatus*[c] | 10 | 6 | 8.2 | 0.96 | | | |
| | 20 | 6 | 17.1 | 1.39 | | | |
| | 50 | 4 | 42.8 | 2.66 | | | |
| *D. magna*[c] | 1 | 4 | 1.0 | 0.02 | | | |
| | 5 | 4 | 4.1 | 0.27 | | | |
| | 10 | 4 | 9.9 | 0.24 | | | |
| | 20 | 2 | 17.9 | 1.27 | | | |

[a]Total concentration of all compounds as measured by HPLC.

[b]Number of samples analyzed.

[c]Replicate treatment group A and B were exposed in separate "egg cups" suspended in the same exposure tank.

Table 30. SUMMARY OF WATER QUALITY MEASUREMENTS PERFORMED DURING THE FLOW-THROUGH ACUTE TESTS ON 2,4-DNT

| Test Species/ Parameter Statistics | Temperature (°C) | Dissolved Oxygen (mg/L) | pH | Hardness (mg/L as $CaCO_3$) | Alkalinity (mg/L as $CaCO_3$) | Conductance ($\mu$mhos/cm$^2$) |
|---|---|---|---|---|---|---|
| Fathead minnow | | | | | | |
| N[a] | 2 | 12 | 12 | 1 | 1 | 1 |
| Mean | 20.5 | 8.4 | 7.2 | 31 | 30 | 84 |
| Range | 20.0-21.0 | 7.9-8.8 | 7.0-7.4 | -- | -- | -- |
| Bluegill | | | | | | |
| N | 4 | 12 | 12 | 1 | 1 | 1 |
| Mean | 20.8 | 7.9 | 7.2 | 27 | 20 | 58 |
| Range | 20.0-21.0 | 6.4-8.7 | 7.0-7.5 | -- | -- | -- |
| Channel catfish | | | | | | |
| N | 2 | 12 | 12 | 1 | 1 | 1 |
| Mean | 20.0 | 7.6 | 7.1 | 25 | 40 | 65 |
| Range | 0 | 5.8-8.3 | 6.5-7.4 | -- | -- | -- |
| Rainbow trout | | | | | | |
| N | 12 | 9 | 9 | 1 | 1 | 1 |
| Mean | 13.0 | 9.5 | 7.6 | 17 | 30 | 27 |
| Range | 0 | 8.4-9.9 | 6.9-8.0 | -- | -- | -- |
| D. magna | | | | | | |
| N | 2 | 10 | 10 | 1 | 1 | 1 |
| Mean | 20.0 | 8.3 | 7.5 | 20 | 25 | 55 |
| Range | 0 | 8.1-8.6 | 7.1-7.8 | -- | -- | -- |
| L. variegatus | | | | | | |
| N | 2 | 12 | 12 | 1 | 1 | 1 |
| Mean | 21 | 8.2 | 7.3 | 45 | 55 | 125 |
| Range | 0 | 7.7-8.5 | 7.0-7.6 | -- | -- | -- |

[a]Number of measurements performed.

Table 31. SUMMARY OF WATER QUALITY MEASUREMENTS PERFORMED DURING THE FLOW-THROUGH ACUTE TESTS ON CONDENSATE WATER

| Test Species/ Parameter Statistics | Temperature (°C) | Dissolved Oxygen (mg/L) | pH | Hardness (mg/L as $CaCO_3$) | Alkalinity (mg/L as $CaCO_3$) | Conductance ($\mu$mhos/cm$^2$) |
|---|---|---|---|---|---|---|
| Fathead minnow | | | | | | |
| N[a] | 2 | 12 | 12 | 1 | 1 | 1 |
| Mean | 21.0 | 8.4 | 7.8 | 41 | 50 | 104 |
| Range | 0 | 7.8-8.6 | 7.6-8.5 | -- | -- | -- |
| Bluegill | | | | | | |
| N | 4 | 12 | 12 | 1 | 1 | 1 |
| Mean | 20.2 | 7.9 | 7.5 | 43 | 70 | 110 |
| Range | 20.0-21.0 | 7.3-8.2 | 7.3-7.8 | -- | -- | -- |
| Channel catfish | | | | | | |
| N | 2 | 9 | 9 | 1 | 1 | 1 |
| Mean | 20.5 | 7.1 | 7.8 | 76 | 70 | 192 |
| Range | 20.0-21.0 | 5.5-7.7 | 7.6-8.1 | -- | -- | -- |
| Rainbow trout | | | | | | |
| N | 4 | 9 | 9 | 1 | 1 | 1 |
| Mean | 14.2 | 9.8 | 7.9 | 76 | 70 | 192 |
| Range | 13.0-15.5 | 8.1-10.8 | 7.6-8.2 | -- | -- | -- |
| D. magna | | | | | | |
| N | 2 | 12 | 12 | 1 | 1 | 1 |
| Mean | 20.5 | 8.4 | 7.8 | 45 | 55 | 97 |
| Range | 20.0-21.0 | 7.9-8.5 | 7.6-8.0 | -- | -- | -- |
| L. variegatus | | | | | | |
| N | 1 | 10 | 4 | 1 | 1 | 1 |
| Mean | 22.0 | 8.4 | 8.0 | 71 | 80 | 190 |
| Range | -- | 8.2-8.7 | 7.9-8.0 | -- | -- | -- |

[a]Number of measurements performed.

Table 32. ACUTE TOXICITY OF 2,4-DNT TO SELECTED SPECIES OF FISH AND INVERTEBRATES EXPOSED UNDER FLOW-THROUGH CONDITIONS

| Test Species | Pooled LC50 (mg/liter)[a] 24-hr | 48-hr | 96-hr | Incipient[b] | | 95% Confidence Limits for the Incipient LC50 | | |
|---|---|---|---|---|---|---|---|---|
| Fathead minnow | 37.7 | 37.7 | 36.1 | 26.0 | (336) | 23.4 | - | 28.5 |
| Bluegill | 30.2 | 29.9 | 16.0 | 9.2 | (336) | 8.5 | - | 10.4 |
| Channel catfish | 36.7 | 33.8 | 32.0 | 16.4 | (336) | 14.1 | - | 18.9 |
| Rainbow trout | 19.6 | 19.3 | 13.9 | 6.3 | (336) | 5.6 | - | 7.0 |
| Daphnia magna | 31.2 | 30.4 | 23.9 | 4.1 | (288)[c] | 3.0 | - | 5.3 |
| L. variegatus | 99.5 | 80.9 | 47.2 | 30.4 | (336) | 25.8 | - | 35.8 |

[a]Based on measured concentrations.

[b]Total hours of exposure shown in parentheses.

[c]Control mortality was 10.0 percent.

Table 33. ACUTE TOXICITY OF CONDENSATE WATER (FINAL BLEND) TO SELECTED SPECIES OF FISH AND INVERTEBRATES EXPOSED UNDER FLOW-THROUGH CONDITIONS

| Test Species | Pooled LC50 (mg/liter)[a] 24-hr | 48-hr | 96-hr | Incipient[b] | | 95% Confidence Limits for the Incipient LC50 | | |
|---|---|---|---|---|---|---|---|---|
| Fathead minnow | 13.4 | 9.8 | 9.3 | 5.9 | (312) | 5.3 | - | 6.7 |
| Bluegill | 14.5 | 6.3 | 4.1 | 2.8 | (288) | 2.2 | - | 3.5 |
| Channel catfish | 12.4 | 10.3 | 8.0 | 7.3 | (192) | 6.5 | - | 8.2 |
| Rainbow trout | 10.7 | 7.5 | 7.3 | 2.3 | (336) | 1.0 | - | 5.3 |
| Daphnia magna | >17.9 | >17.9 | 9.2 | 8.5 | (144)[c] | 7.2 | - | 10.0 |
| L. variegatus | >42.8 | 33.8 | 18.9 | 17.6 | (192) | 14.5 | - | 21.6 |

[a]Based on measured concentrations.

[b]Total hours of exposure shown in parentheses.

[c]Control mortality was 13.3 percent.

# CONCLUSIONS AND RECOMMENDATIONS

## Conclusions

Based on the results of the 14-day, flow-through acute toxicity tests, condensate wastewater could cause acute effects in some aquatic species if the total concentration of its organic components in bodies of water that receive the wastewater exceeds 2 mg/L for approximately 10 days or 5 mg/L for approximately 2 days. The results of the algal assays indicate that the wastewater may cause growth inhibition in certain species of algae if the concentration of 2,4-DNT exceeds 0.5 mg/L or the total concentration of the organic components of the wastewater exceeds 1.0 mg/L. Effects at that level could become apparent within 96 hours.

Some of the components of condensate wastewater are highly toxic to aquatic life. The most toxic of those tested is 2,3,6-trinitrotoluene. Other highly toxic components (48- or 96-hr LC50, 1 mg/L or less) are 2-amino-3,6-dinitrotoluene and 1,3,5-trinitrobenzene. Because they are photolabile, 2,3,6-trinitrotoluene, 2-amino-3,6-dinitrotoluene, and the majority of the other organic components of the wastewater are unlikely to persist, except, possibly near the discharge site, at concentrations high enough to cause severe chronic problems. However, if wastewater discharge is continuous, the concentrations of the photolabile compounds could be maintained at a level high enough to cause chronic problems at or near the discharge site. The area affected would depend primarily on the initial concentrations and the rates of dilution and photolysis.

Of all the compounds tested, 1,3,5-trinitrobenzene was not only one of the most acutely toxic, but it was also resistant to phototransformation. Also, there is evidence that it might be a reaction or transformation product of other condensate wastewater components. Field studies have shown that its concentration in receiving waters is higher than its concentration in condensate wastewater, and our study showed that its concentration in the wastewater increases when the wastewater is exposed to filtered UV light. This compound may pose a chronic hazard. Computed Log P and BCF values for this compound indicate that its propensity to bioconcentrate is low, however. Other non-photoreactive compounds present in the wastewater at concentrations greater than one-tenth of their estimated LC50s are 1,3-dinitrobenzene, 3,4-dinitrotoluene, and 3-amino-2,4-dinitrotoluene. These compounds could also present a long-term chronic toxicity hazard.

The compound 2,4-DNT accounts for about 44 percent of the total concentration of the nitroaromatic constituents and other organic by-products of TNT manufacture in condensate wastewater. Except for certain species of algae, its acute toxicity to aquatic organisms appears to be relatively low. It is also photolabile.

Seasonal and other variations in the temperature, pH, and hardness of receiving waters should have little effect on the acute toxicity of condensate wastewater. Exposure to sunlight should reduce its acute toxicity.

It is unlikely that any of the organic constituents of condensate wastewater will bioconcentrate extensively.

## Recommendations

Field studies at selected ammunition plants have provided evidence that aquatic organisms living in receiving waters with munition compound concentrations as low as 50$\mu$g/L may be affected by these compounds or by non-munitions materials also present in the effluent. Although none of the materials reported on in this volume caused acute effects at levels as low as those found in the field studies, the levels of effects observed in the laboratory studies were not sufficiently high to exclude munitions-related materials as a cause of concern. Therefore, we recommend the conduct of longer-term exposure studies to determine the concentrations that do and do not cause chronic effects. We also recommend the evaluation of 1,3,5-trinitrobenzene for chronic toxicity.

## LITERATURE CITED


ASTM. 1978. Estimating the Hazard of Chemical Substances to Aquatic Life. J. Cairns Jr., K.L. Dickson, and A.W. Maki, eds. Amer. Soc. Test. Materials. STP 657. 278 pp.

Bakke, T. and H.R. Skjoldal. 1979. The effects of toluene on the survival, respiration, and adenylate system of a marine isopod. Marine Pollution Bulletin 10, 111-115.

Bandt, H.J. 1946. Damage to the taste of fish. Beitr. Wass. Abwass. Fischer-ichem 1:36.

Berry, W.O. and J.D. Brammer. 1977. Toxicity of water-soluble gasoline fractions to fourth-instar larvae of the mosquito Aedes aegypti. Environ. Pollut. 13, 229-234.

Bringman, G. and R. Kuhn. 1959. The toxic effects of wastewater on aquatic bacteria, algae, and small crustaceans. Gesundheits-Ing. 80:115.

Bringmann, G. and R. Kuhn, 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Research 14, 231-241.

Burton, D.T. 1972. Evaluation of Radford Army Ammunition Plant's major water outfalls by acute bioassay procedures. In Biological and Engineering Investigation to Develop Optimum Control Measures to Prevent Water Pollution. L.L. Smith and W.I. Dickerson (eds.). RAAP, Final Engineering Report, Production Engineering *Project* PE-249 (Phase I), Propellant Plant Pollution Abatement.

Degani, J.B. 1943. Studies on the toxicity of ammunition plant wastes. Transact. Amer. Fish. Soc. 73:45.

Environmental Protection Agency. 1971. Algal Assay Procedures: Bottle Test. National Eutrophication Research Program. Corvallis, Oregon. 82 pp.

Environmental Protection Agency. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series. 660/3-75-009. 61 pp.

Korn, S., D.A. Moles, and S.D. Rice. 1979. Effects of temperature on the median tolerance limit of pink salmon and shrimp exposed to toluene, naphthalene, and Cook Inlet crude oil. Bull. Environm. Contam. Toxicol. 21, 521-525.

LeClerc, E. 1960. The self-purification of streams and the relationship between chemical and biological tests. Proc. Second Symp. on Treatment of Wastewater. p. 281.

Leo, A., C. Hanch, and D. Elkins. 1971. Partition coefficients and their uses. Chem. Rev. 71:525.

Liu, D.H.W., R.J. Spanggord, H.C. Bailey, H.S. Javitz, and D.C.L. Jones. 1980. Toxicity of TNT Wastewater to Aquatic Organisms. Volume I. Acute Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene. Final Report. USAMRDC Contract No. DAMD17-75-C-5056. 106 pp.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria. Second Ed. Resources Agency of California, State Water Resources Control Board, Publication No. 3-A. p. 287.

Ohmori, S., M. Ikeda, and M. Ogata. 1975. The metabolism and accumulation of petroleum components in fish: the side chain oxidation of p-nitrotoluene and p-nitrobenzyl alchohol in liver homogenates of the rat and eel. Physiol. Chem. and Physics 7, 477-480.

Pearson, J.G., J.P. Glennon, J.J. Barkley, and J.W. Highfill. 1979. An approach to the toxicological evaluation of a complex industrial wastewater. In: Aquatic Toxicology, ASTM STP 667. L.L. Marking and R.A. Kimmerle, Eds. Amer. Soc. Test. Materials, pp. 284-301.

Pickering, Q.H. and C. Henderson. 1966. Acute toxicity of some important petrochemicals to fish. J. Water. Poll. Contr. Fed. 38:1419.

Schott, C.D. and E.G. Worthley. 1974. The toxicity of TNT and related wastes to an aquatic flowering plant, Lemna perpusilla Torr. Defense Technical Information Center, Report No. AD-778158, Edgewood Arsenal, Aberdeen Proving Ground, Maryland.

Spanggord, R.J., B.W. Gibson, R.G. Keck, and G.W. Newell. 1978. Mammalian Toxicological Evaluation of TNT Wastewater (Pink Water). Volume I. Chemistry Studies. Final Report. USAMRDC Contract No. DAMD17-26-C-6050. 315 pp.

Spanggord, R.J., T. Mill, T.W. Chou, W.R. Mabey, J.H. Smith, and S. Lee. 1980. Environmental fate studies on certain munition wastewater constituents. Final Report, Phase II, USAMRDC Contract No.DAMD17-78-C-8081.

Sprague, J.B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Research 4:3.

Sprague, J.B. and A.B. Ramsey. 1965. Lethal levels of mixed copper-zinc solutions for juvenile salmon. J. Fish. Res. Bd. Can. 22:425.

Sullivan, J.H., Jr., H.D. Putnam, M.A. Keirn, D.R. Swift, and B.C. Pruitt, Jr. 1977a. Aquatic Field Surveys at Volunteer Army Ammunition Plant, Chattanooga, Tennessee. Final Report. USAMRDC Contract No. DAMD17-75-C-5049. 316 pp.

Sullivan, J.H., Jr., H.D. Putnam, M.A. Keirn, D.R. Swift, and B.C. Pruitt, Jr. 1977b. Aquatic Field Surveys at Holston Army Ammunition Plant, Kingsport, Tennessee. Final Report, USAMRDC Contract No. DAMD17-75-C-5049. 266 pp.

Veith, G. et al. 1979. Measuring and estimating the bioconcentration factors of chemicals in fish. J. Fish. Res. Bd. Can. 36:1040.

Wallen, I.E., W.C. Greer, and R. Lasater. 1957. Toxicity to Gambusia affinis of certain pure chemicals in turbid waters. Sewage and Indust. Wastes. 29:695.

Wentsel, R.S., R.G. Hyde, W.E. Jones, III, M.J. Wilkinson, W.E. Harward, III, and J.F. Kitchens. 1979. Problem Definition study on 1,3-dinitrobenzene, 1,3,5-trinitrobenzene and di-n-propyl adipate. Final Report Contract No. DAMD17-77-C-7057. U.S. Army Medical Research and Development Command, Fort Detrick, Maryland.

DISTRIBUTION LIST

| | |
|---|---|
| 25 copies | Commander<br>US Army Medical Bioengineering<br>Research and Development Laboratory<br>ATTN: SGRD-UBG<br>Fort Detrick<br>Frederick, MD 21701 |
| 4 copies | USAMRDC (SGRD-RMS)<br>Fort Detrick<br>Frederick, MD 21701 |
| 12 copies | Defense Technical Information Center (DTIC)<br>ATTN: DTIC-DDA<br>Cameron Station<br>Alexandria, VA 22314 |
| 1 copy | Dean<br>School of Medicine<br>Uniformed Services University of the<br>Health Sciences<br>4301 Jones Bridge Road<br>Bethesda, MD 20014 |
| 1 copy | Commandant<br>Academy of Health Sciences, US Army<br>ATTN: AHS-CDM<br>Fort Sam Houston, TX 78234 |
| 1 copy | Commander<br>US Army Medical Bioengineering<br>Research and Development Laboratory<br>ATTN: SGRD-UBD-A/Librarian<br>Fort Detrick<br>Frederick, MD 21701 |